Animal models in biofilm and CF research.

Medical Biofilm Techniques Course 2009

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Take home messages

- Biofilm infections are numerous and they are a daily challenge, especially in the hospitals.
- Representative (animal) models are mandatory.
- Several animal models are available. Constantly being improved and adjusted to relevant problem of interest.
- Frequent contact between the clinical world and the basic science is important.

Clinical introduction.

Different forms?

- Foreign body associated biofilm infections.
  - External
  - Internal
- Non-foreign body associated biofilm infections.
  - Tissue adhering
  - Free biofilms

Foreign body associated biofilm infections

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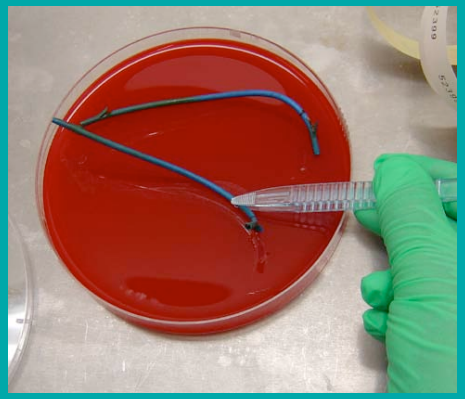
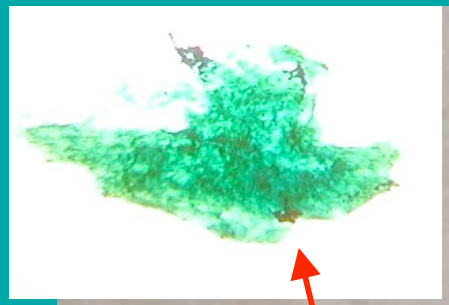
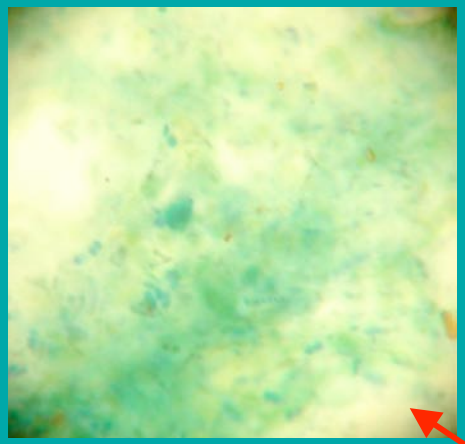
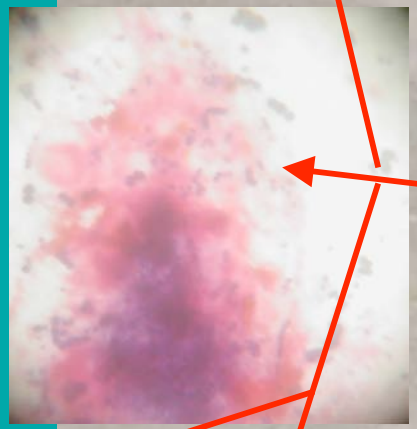
Foreign body associated biofilm infections.

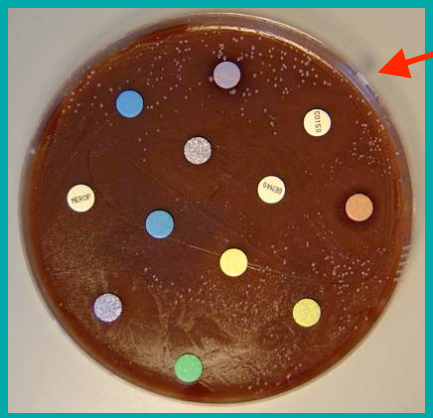
- External
  - Blood stream catheters (+ tunnelated). (Guo B, et al. IAI 2007. Shuford, JA, et al JID 2006).
  - Urinary tract catheters. (Goto T, et al. Int J Antimicrob Agents 1999).
  - Intraperitoneal dialysis catheters.
  - Ventilation tubes. (Luna CM, et al. Chest 2007).
  - Drainage tubes.
  - Lenses.
- Internal
  - Organ stents. (Minardi et al. Peptides. 2007).
  - Blood stream stents/Pacemakers.
  - Arthroplasty/osteosynthetic material. (Antoci V Jr, et al. Clin Ortho Rel Res 2007).
  - Intracerebral shunts.
- Many more (Heartmates, mechanical heartvalves, mamma implants, penis implants, intra uterine devices etc, etc)

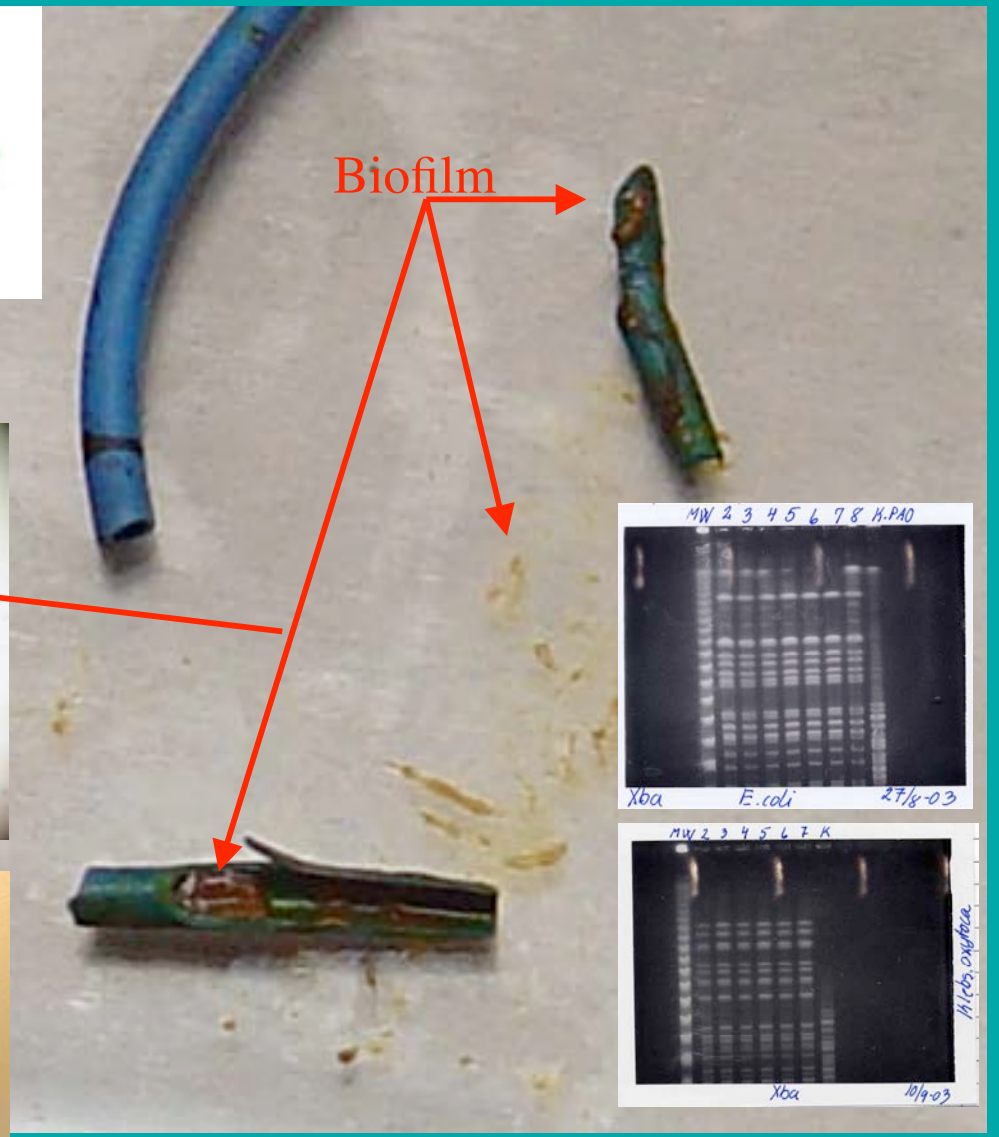












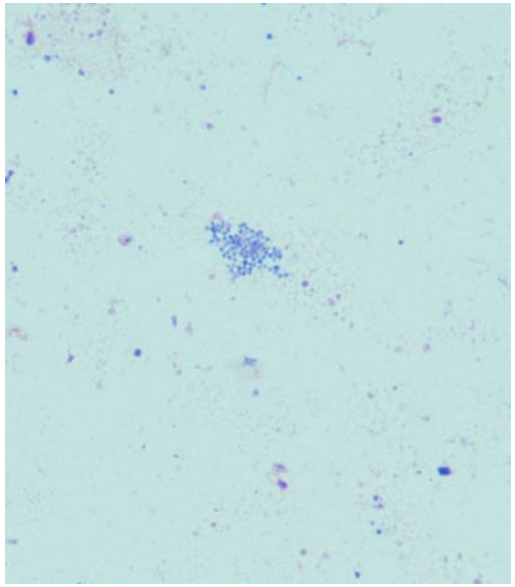
Gram/methylenblåt x 100 & x 1000

57 årig mand L.S., autopsi d. 14-8-03, stent fra galdeveje og pancreas med biofilm, indsat 3-7, focus for E. coli sepsis 21-7 og fatal E. coli + K. oxytoca sepsis 12-8. Vækst fra biofilm: Klebsiella oxytoca, E. coli af samme genotype som i blodet, Enterococcus faecium

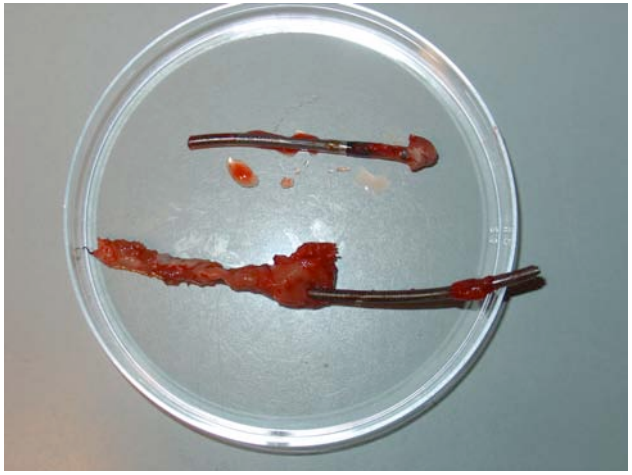
Internal foreign bodies

Coagulase negative staphylococci on

Cerebral shunt



PM electrodes



- 20-year old pt, congenital heartfailure.
- Mechanical hjertevalves and pacemaker.
- Infection with CNS on valves and the pacemakerelectrodes.
- Both had to be changed during extensive heartsurgerystor + long term antibiotic given.

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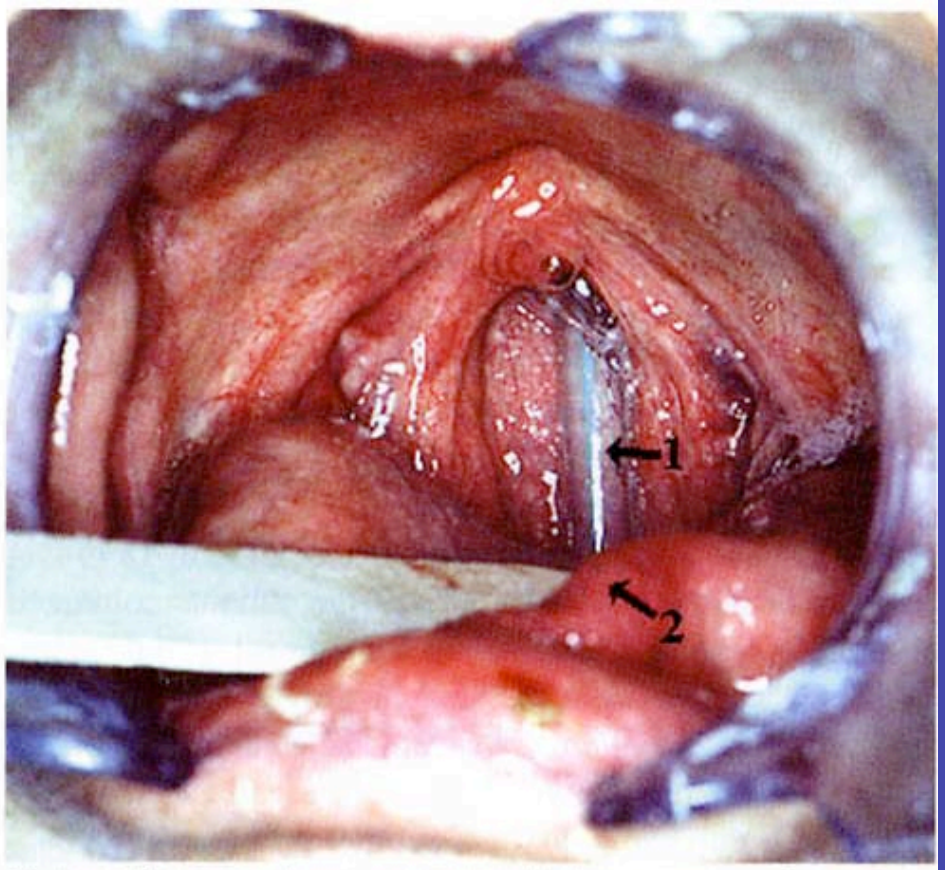


Figure 1. Nasogastric tube embedded in the nasopharynx. 1, nasogastric tube; 2, dorsum of tongue.

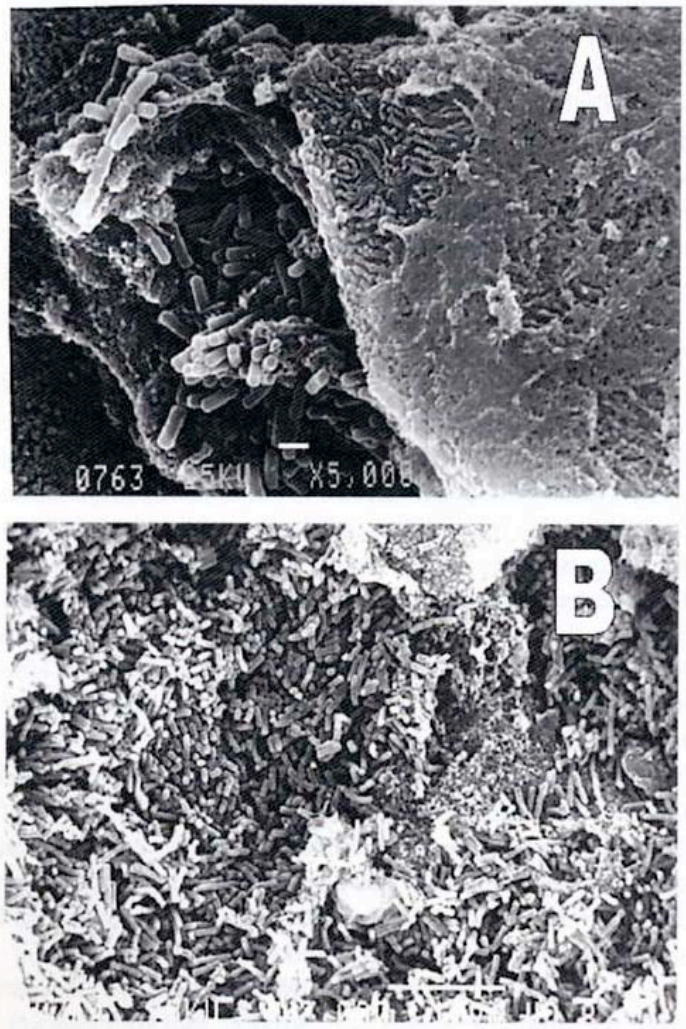
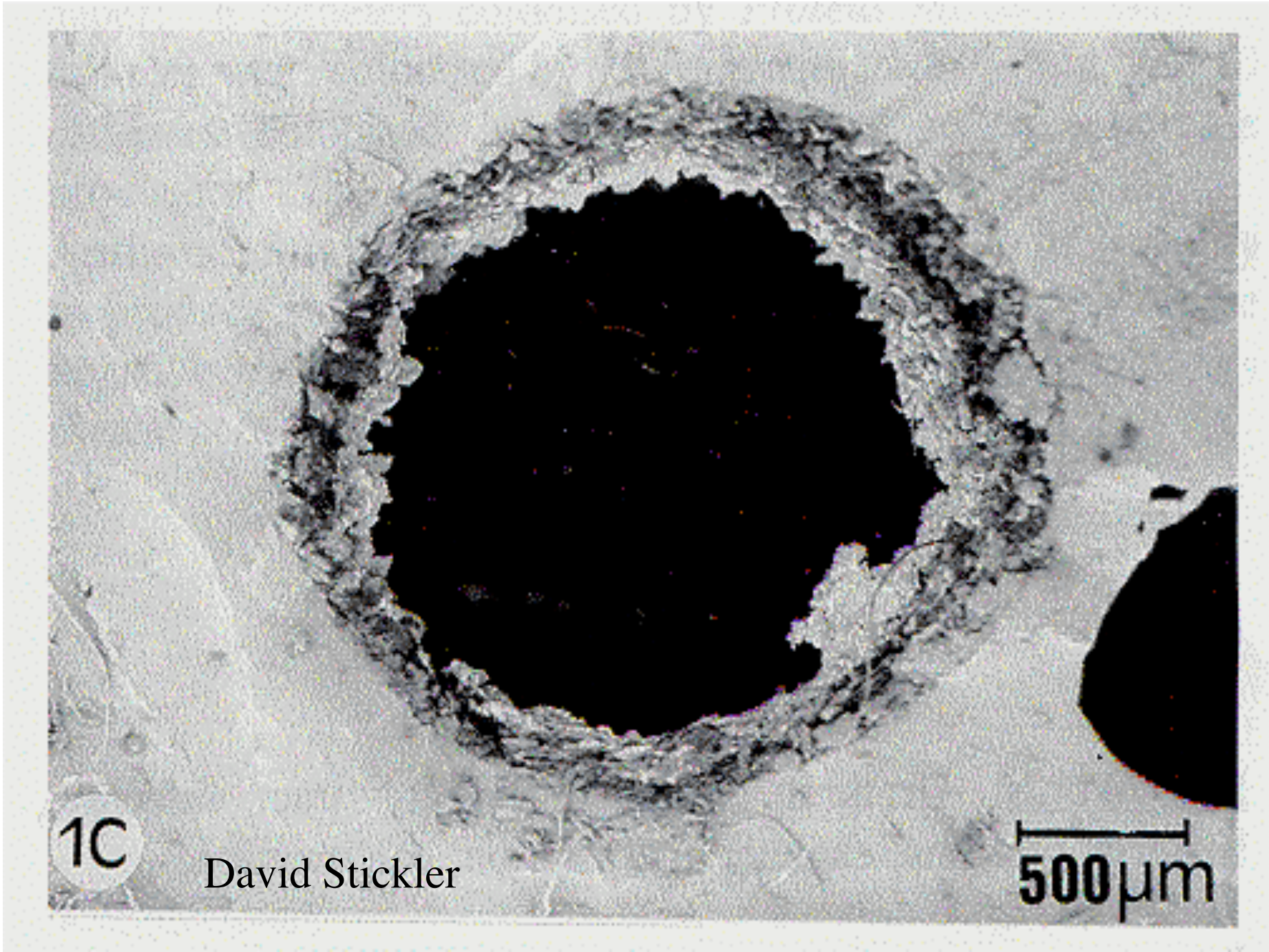


Figure 2. Representative biofilms on nasogastric tubes showing bacterial organisms with typical form of *Pseudomonas aeruginosa*. Scanning electronic microscope. A, scale bar, 1 µm; B, scale bar, 10 µm.

(Leibovitz et al.: P. aeruginosa and the oropharyngeal ecosystem of tube-fed patients. Emerg. Infect. Dis. 9:956-59; 2003)


1C
David Stickler
500µm

Treatment and Consequences. Foreign body associated.

- Generating pathology.
- Often primarily focus for sepsis.
- Early, aggressive treatment.
- Chronic, suppressive treatment.
- Removal of foreign body/implant - often followed by longterm antibiotic treatment.
- Occasionally impossible to re-implant. Can result in amputation, arthrodesis etc.

Non-foreign body associated biofilm infections

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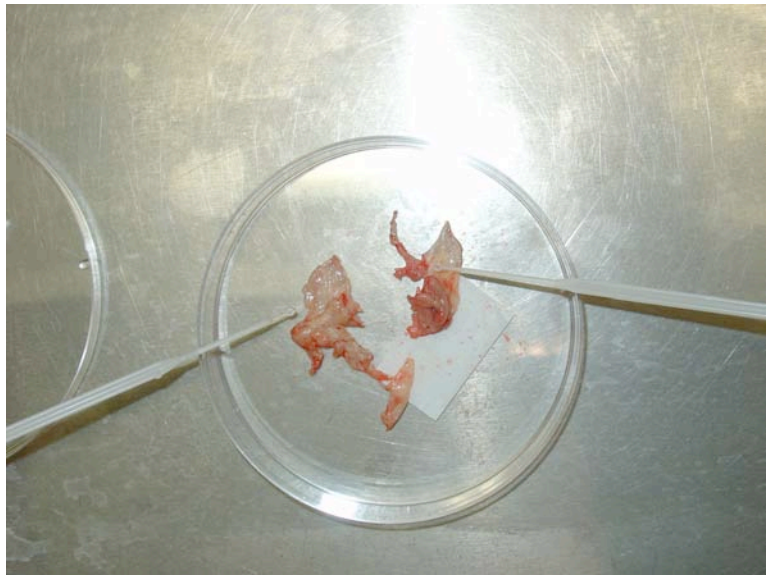
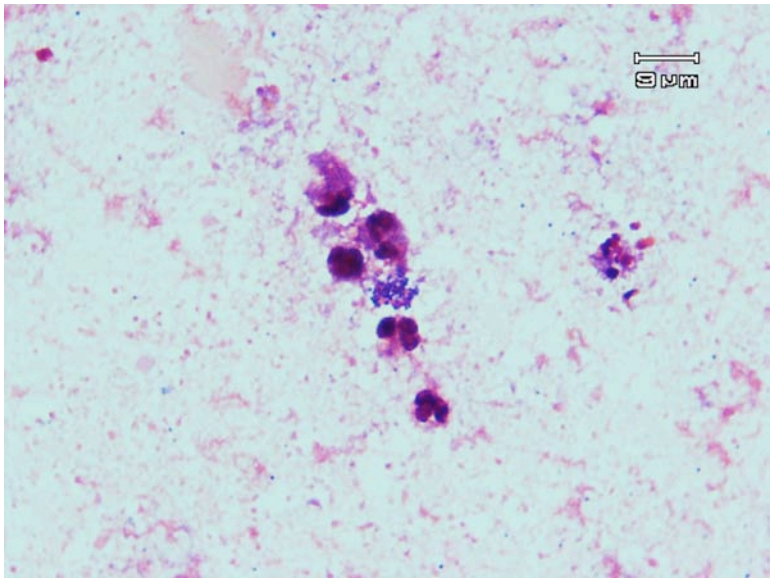
Non-foreign body associated.

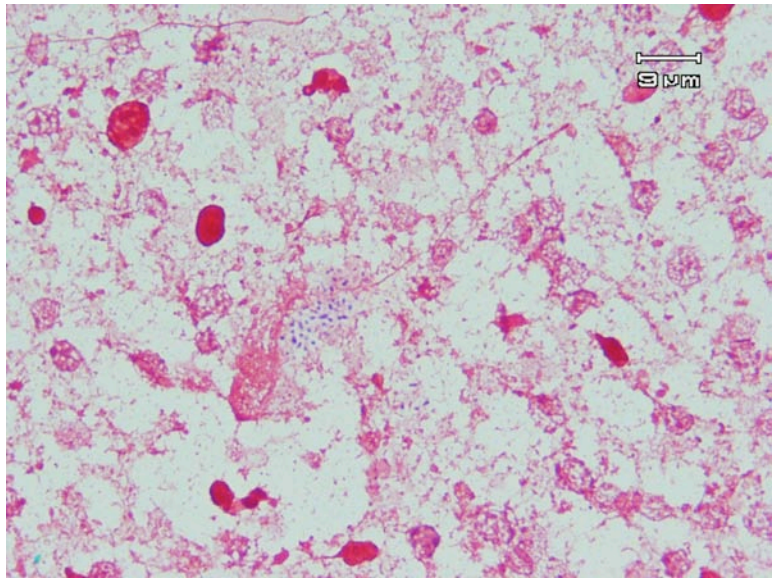
- Chronic lung infections (Cystic fibrosis, COLD, bronchiectasis).
- Chronic otitis media infections. (Leroy M, et al. IAI 2007).
- Chronic urinary tract infections (spinal cord injuries, urolithiasis). (Anderson GG, et al. Trends Microbiol 2004).
- Chronic wounds (Diabetes, artherosclerosis). (Davis SC, et al. Curr Diab Rep 2006).
- Periondontitis (mixed biofilms).
- Chronic sinusitis. (Ha KR, et al. Am J Rhinology 2007).
- Subacute/chronic endocarditis. (Bizzini A, et al. FEMS Immunol Med Microbiol 2006).

Colonizer to pathogen biofilm

S. aureus endokardit







Pneumococci endocarditis

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Biofilm infection?

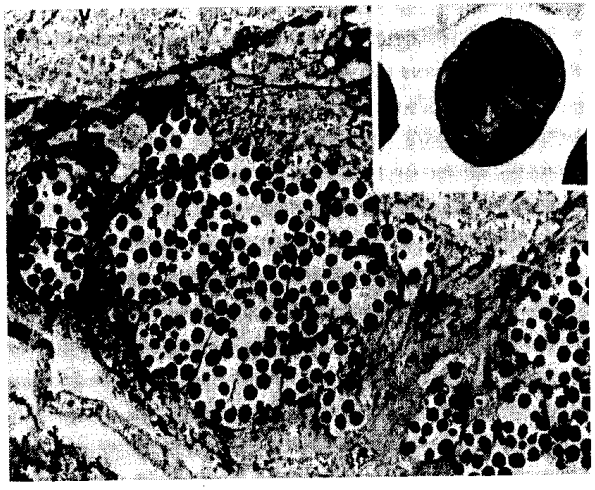
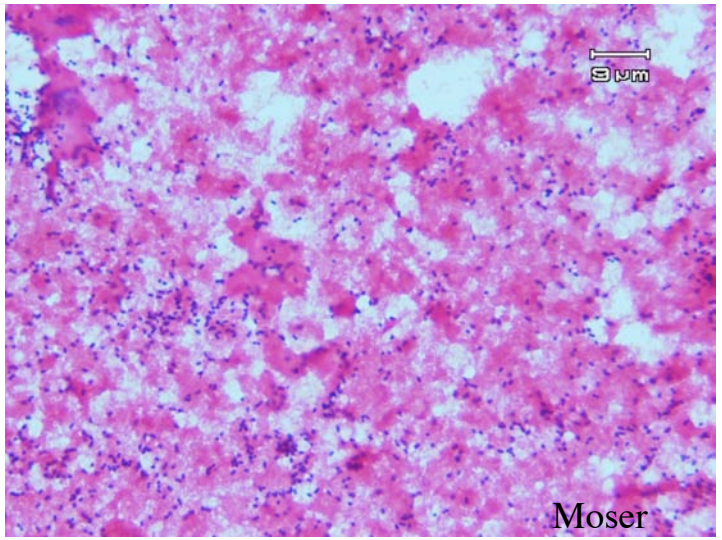
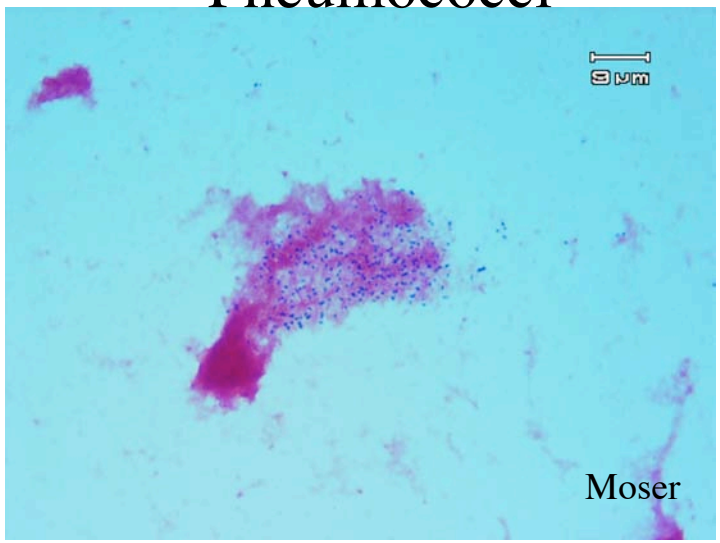


Figure 2 Transmission electron micrograph of a cardiac vegetation. Densely packed rounded staphylococcal microcolonies are seen encased in an extensive matrix probably composed of fibrin strands (×3000). Insert shows a dividing bacterium surrounded by an electron-lucent space that likely resulted from matrix loss during fixation (×30,000). Reproduced with permission from Reference 28a.

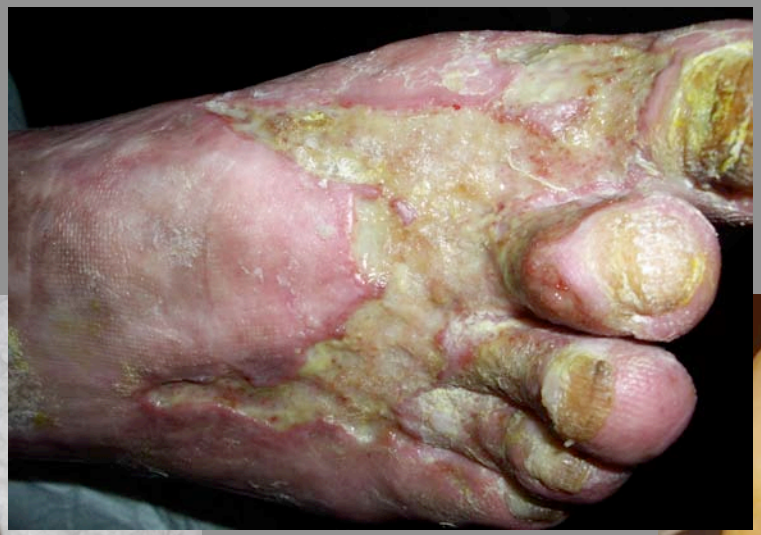
Singh og Parsek 2004

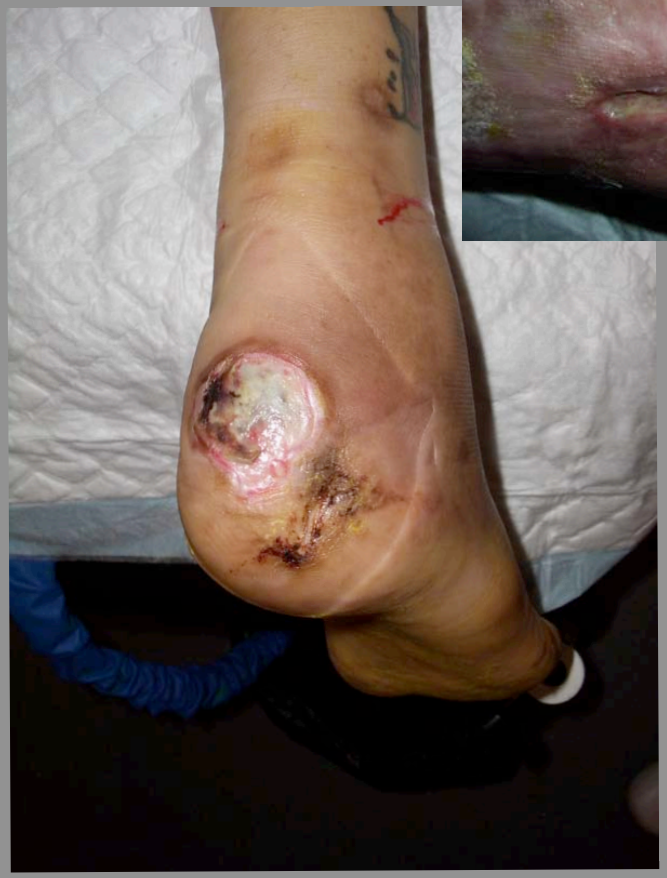


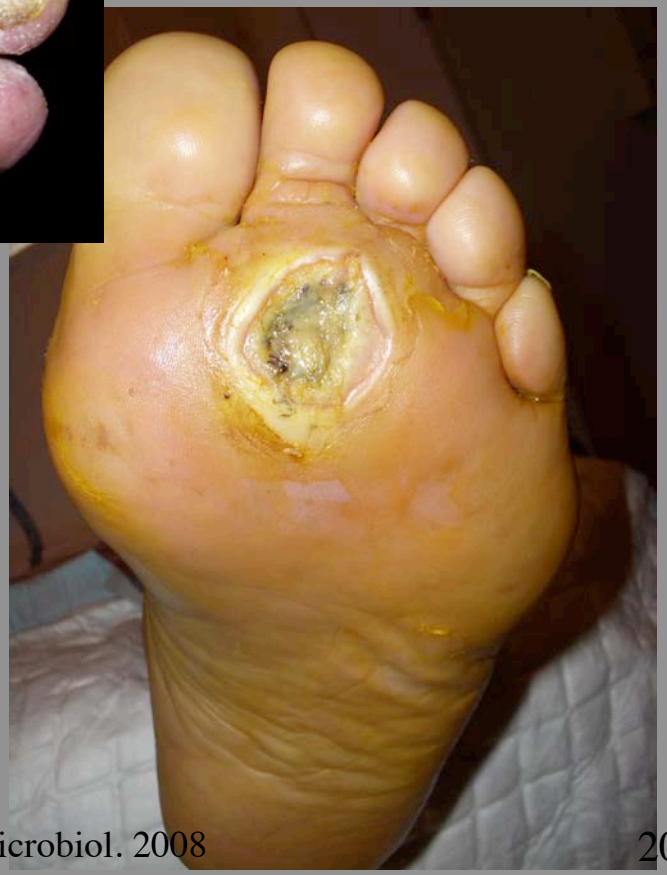
Pneumococci



Chronic Wounds

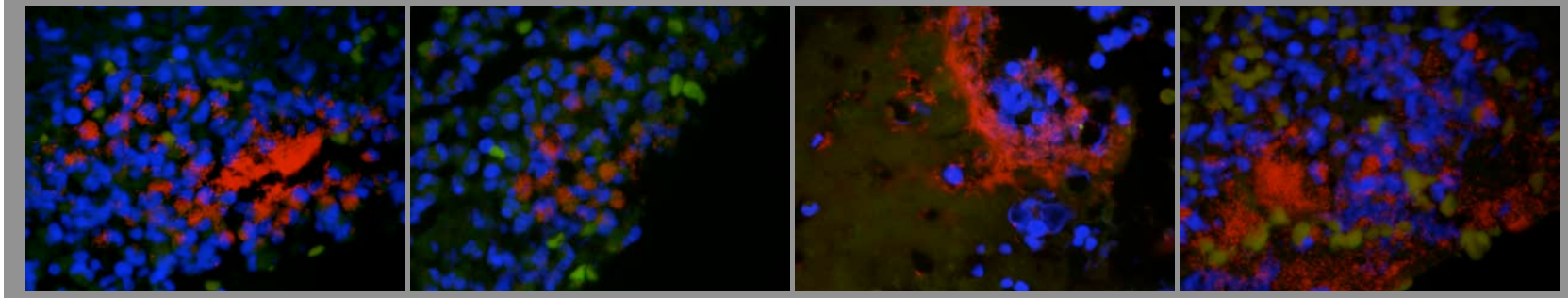




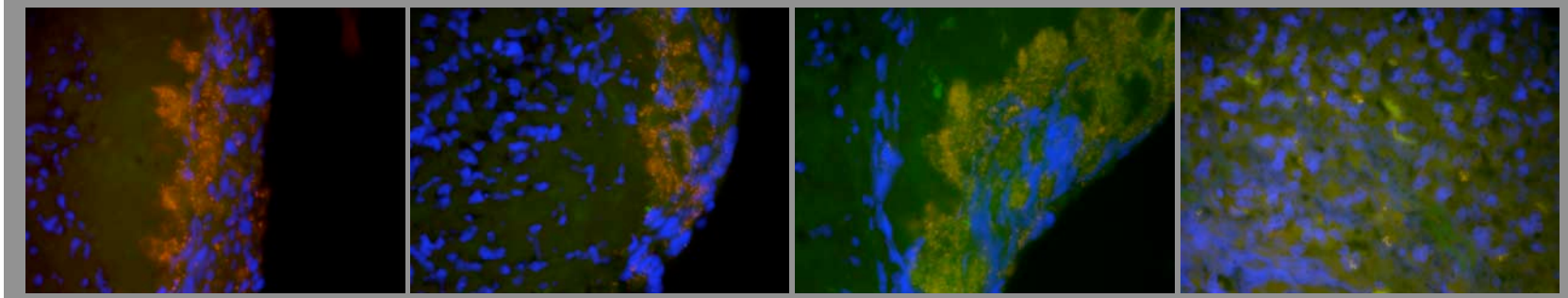


T. Bjarnsholt et al. J Clin Microbiol. 2008

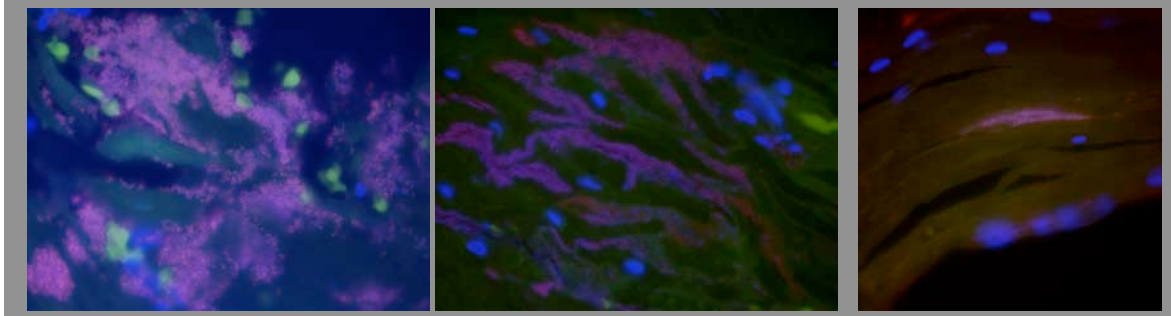
P. aeruginosa specific red



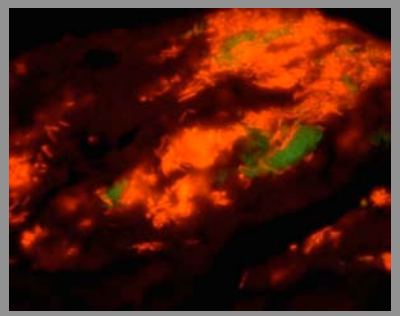
Staph. aureus specific green



Unspecific



mixed



T. Bjarnsholt et al. J Clin Microbiol. 2008

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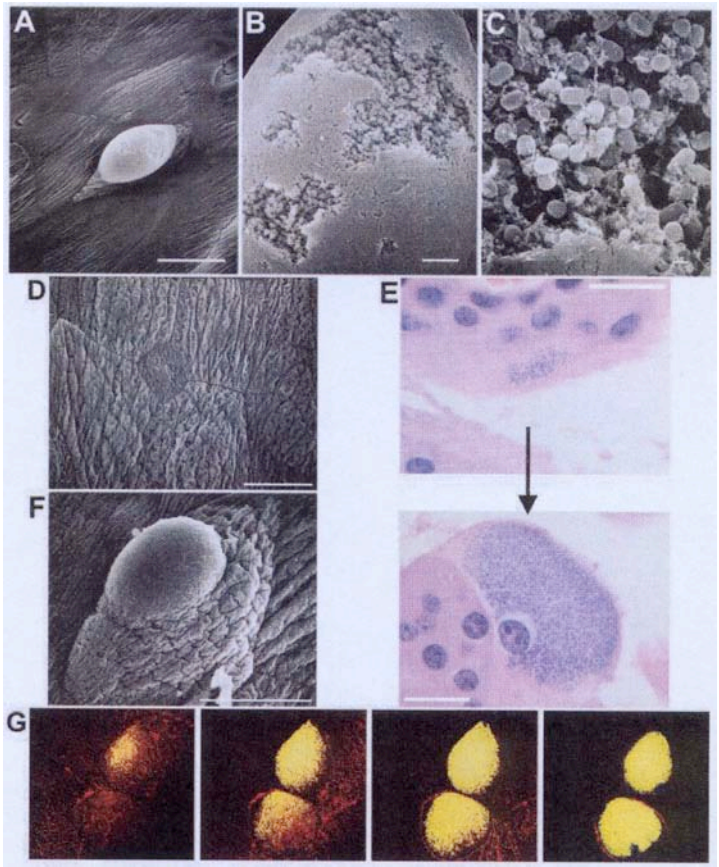
Urinary tract infections



Anderson G et al. Science 2003.

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Treatment and Consequences.

Non-foreign body associated biofilm infections.

- The chronic infection often results in destruction of infected tissue.
- Longterm, high dose, combinatory antibiotic treatment.
- Surgery with removal/replacement of infected tissue can be necessary.
- May lead to other complications, like immune complex disease.

Characteristics

- Persistence.
- Generates pathology.
- Tolerant to antibiotics.
- Tolerant to the immune responses.
- Extracellular matrix.
- Different growth stages?
- Definition: Chronic infection = an infection which A) persists in spite of therapy, and in spite of the host's immune- and inflammatory response and B) (in contrast to colonization) is characterized by persisting pathology and immune response (N. Høiby).

Animal models of biofilm infections - in general.

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The Law (Denmark)

- Animal experiments on vertebrates allowed if the aim is significant, specified and scientific.
- Permission given to individual persons with **sufficient education/background**, after having passed a **special animal experimental course** and test.
- Permission from a council with a professional chairman (Judge) and ten members appointed by the Ministery of Justice.
- Only allowed if other methods cannot replace the experiments.
- Files approved for **all individual** experiments.
- Subject to **unannounced visites** from authorities.
- Annual report from the council in an anonymized form.
- Approximately 300,000 animals used/year in Denmark.
- **Mortality** not allowed as an intended end point.
- Animal wellfare increasing attention.
- **Fanatic anti-animal** experiment groups not a problem in Denmark, so far.

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Number of animals?
(sample size).

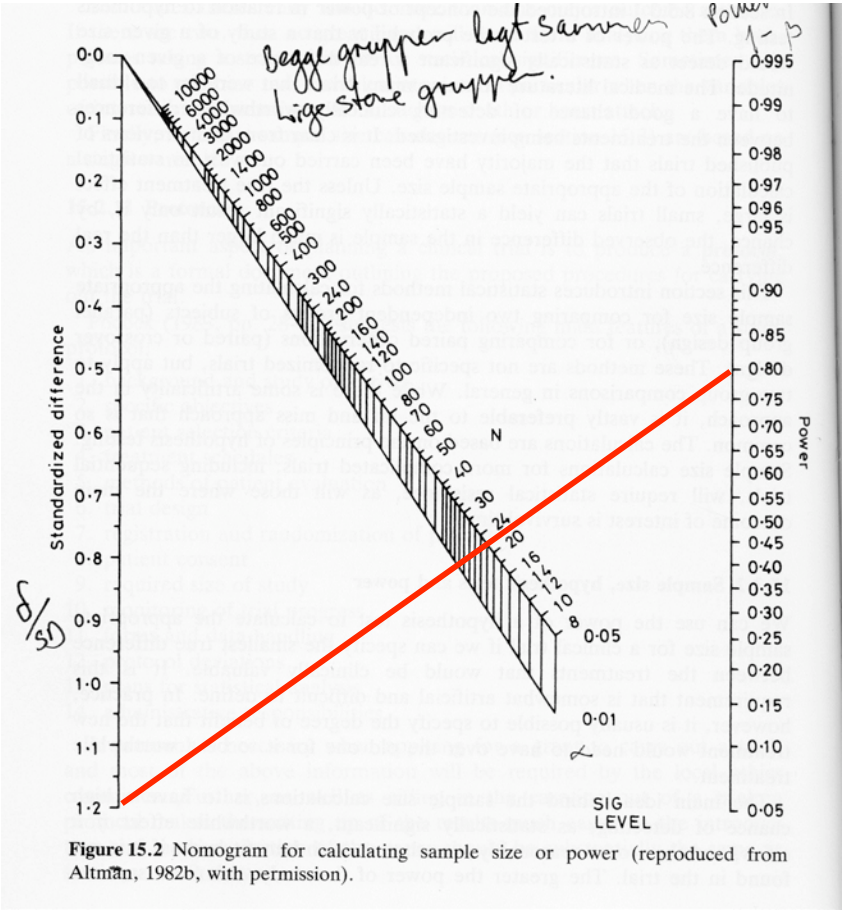

Figure 15.2 Nomogram for calculating sample size or power (reproduced from Altman, 1982b, with permission).

Practical statistics for medical research. Douglas G. Altman. Chapman&Hall. London, UK. 1996.

Total number of animals in two groups.

δ = clinically relevant difference.

SD = standard deviation.

Usually set power (1-β) to 0.80, and α to 0.05.

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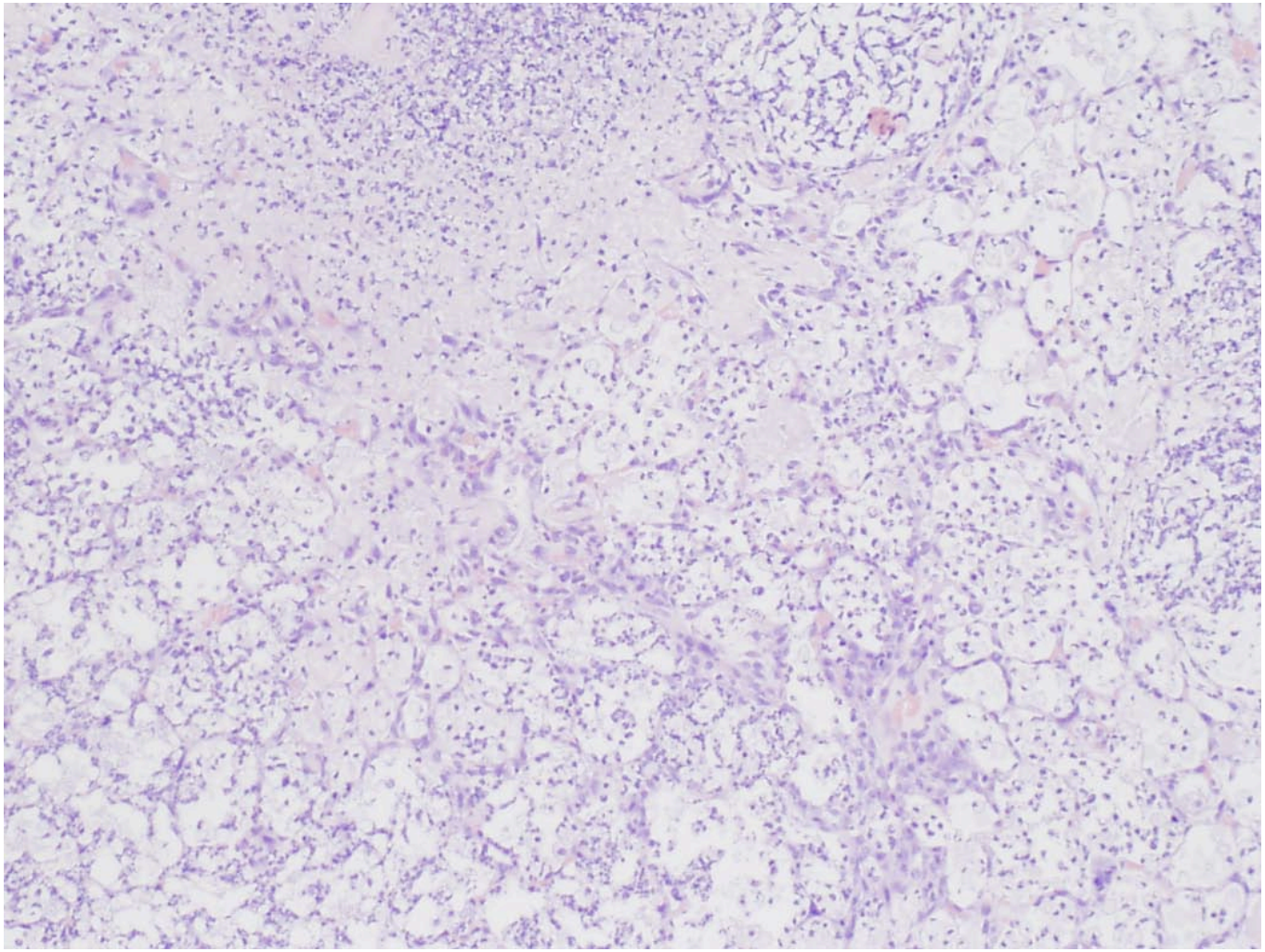
Different microorganisms

- Coagulase negative staphylocci
- *Staphylococcus aureus*
- *Pseudomonas aeruginosa*
- *Escherichia coli*
- *Haemophilus influenzae*
- *Moraxella catarrhalis*
- *Enterococcus faecalis*
- Yeast
- Non-haemolytic streptococci
- Bordetella
- Etc.

Purpose of biofilm infection models

- 1) Consequences of biofilm infection
  - Evaluations by pathology - tissue destruction (macroscopic, histopathology), inflammation, immune response
- 2) Microorganism virulence
  - Use of modulated microorganisms, pathology, culture, visual detection of microorganisms, septic spread
- 3) Prevention of biofilm infection
  - Pretreatment of implant, culture, visual detection of microorganisms
- 4) Treatment of biofilm infection
  - Culture, visual detection of microorganisms, test of organ function

Consequences of biofilm infections - evaluation of pathology: Tissue destruction.



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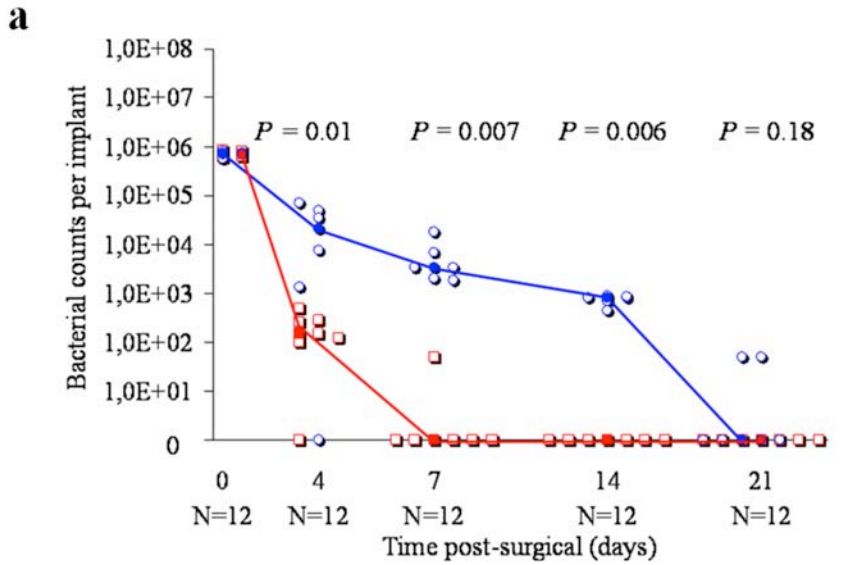
Virulence of Biofilm infection - culture.
Clearing of *P. aeruginosa* wild-type vs. the *ΔlasR rhlR* mutant

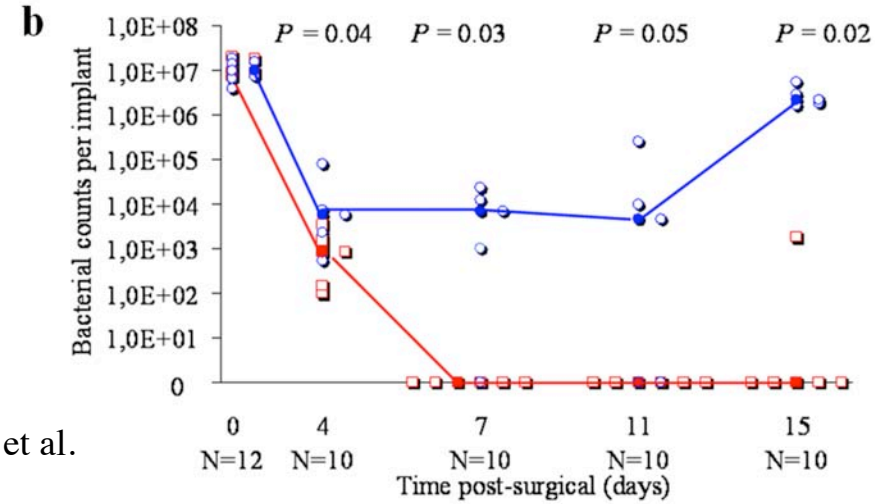
Balb/c mice



Red: *ΔlasR rhlR*

Blue: Wild type

NMRI mice



Louise Dahl Christensen et al.
Microbiology 2007

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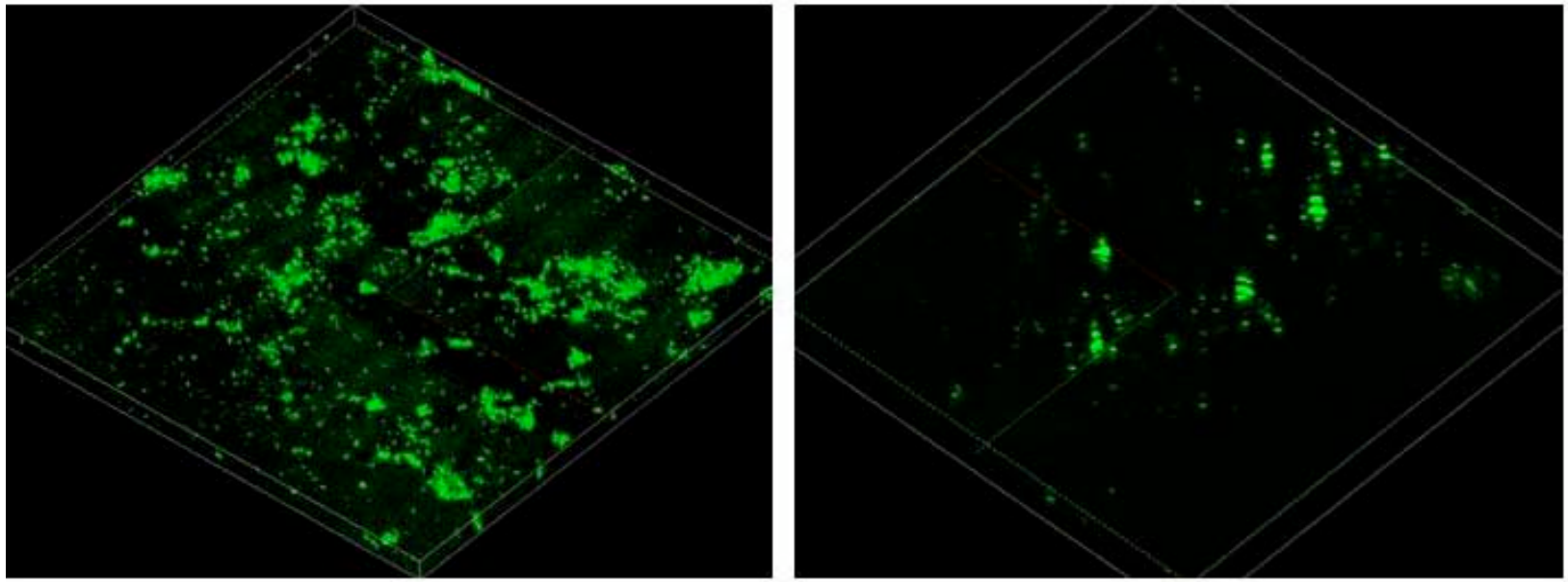
Virulence of Biofilm infection - visual detection (CLSM image) Wild-type and QS mutant

Wild-type *ΔlasR rhlR*



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Prevention of biofilm infection - culture. Prophylaxis of endocarditis.

Non-bacterial thrombotic endocarditis of the aortic valve induced by insertion of a catheter. Prophylaxis 0.5h or 1h prior to i.v. challenge with 10^7 CFU of *S. oralis*.

Table 1. Results of prophylaxis with moxifloxacin in rabbits challenged with *S. oralis*

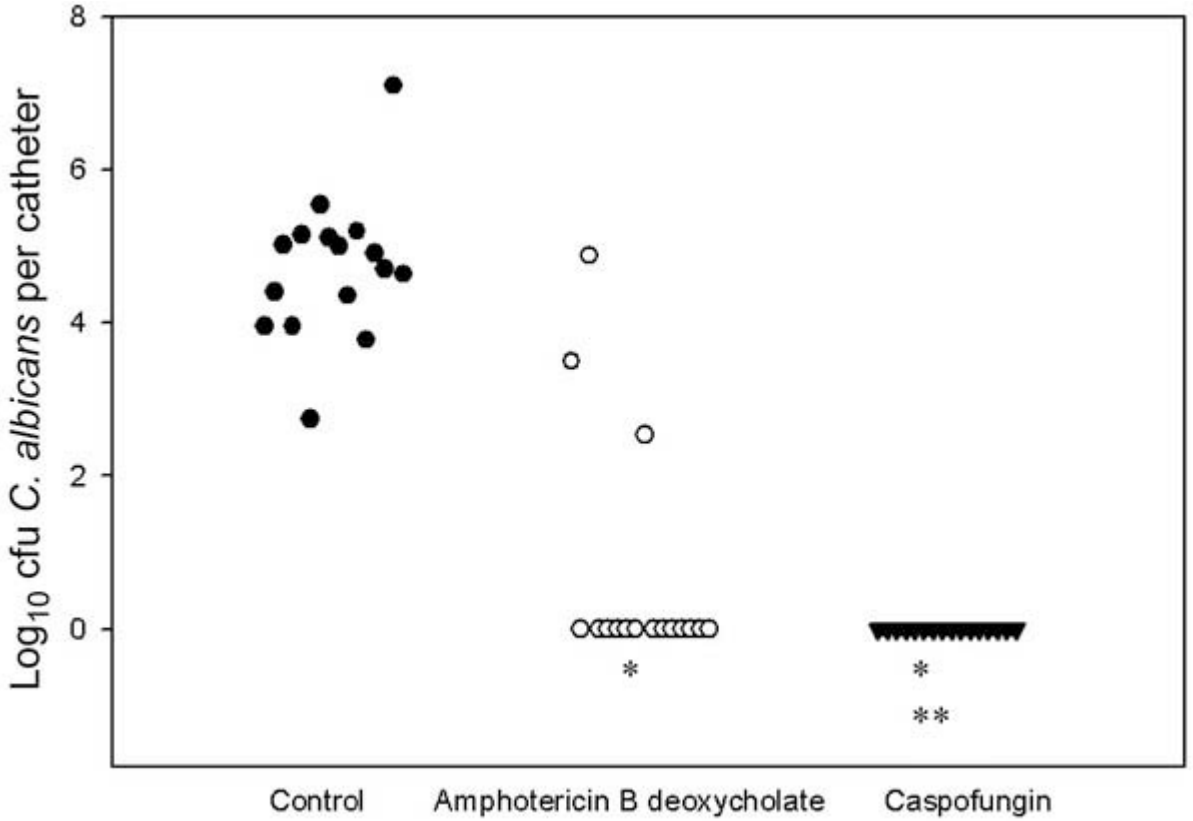
Regimen	No. of vegetations [sterile/total (%)]	Log_{10} cfu/g of non-sterile vegetations (mean ± SD)
No prophylaxis	2/18 (11)	9.21 ± 0.94 (*n* = 16)
Ampicillin	7/14[a] (50)	7.78 ± 2.07 (*n* = 7)
Moxifloxacin	12/15[b] (80)	8.38 ± 0.77 (*n* = 3)

[a] Significantly different from the value obtained for the control group ($P = 0.022$).
[b] Significantly different from the value obtained for the control group ($P < 0.001$).

Sakka V, et al. JAC 2005.

Treatment of biofilm infection - culture.
Treatment of Candida albicans on intravenous catheter.

Rabbits with experimental central venous catheter *Candida albicans* infection.
Treated systemically and by luminal lock (7d) with Amphotericin B or Caspofungin.



Shuford JA, et al. JID 2006.

The peritoneal implant model

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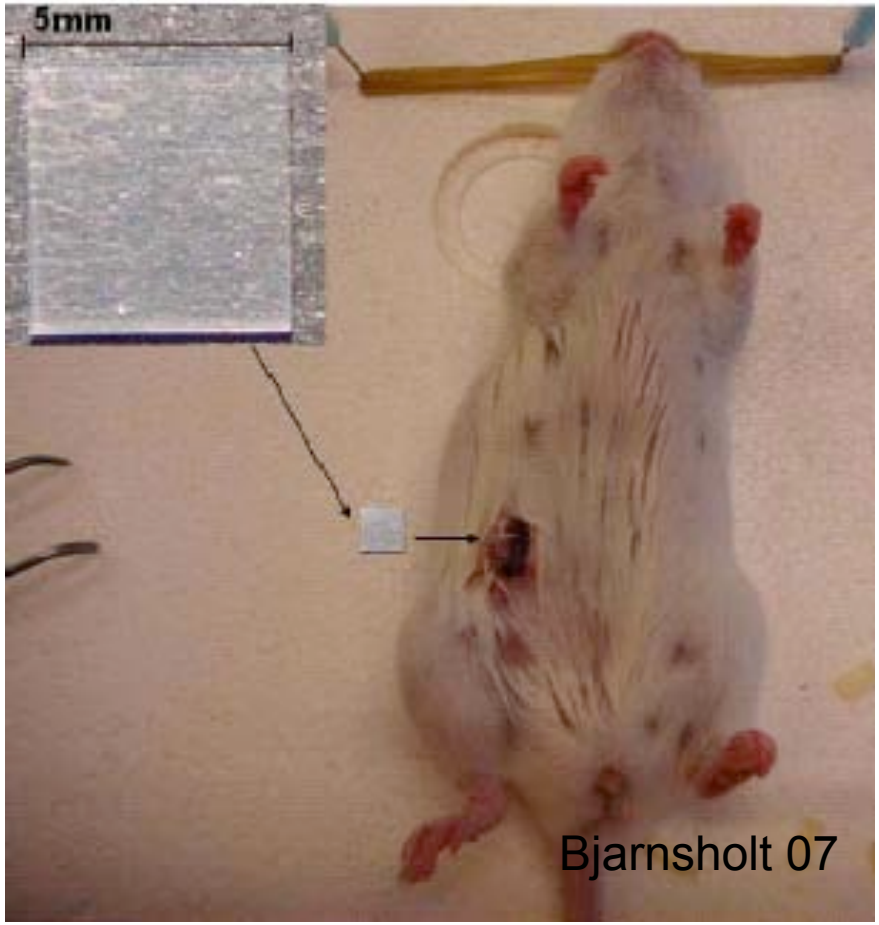
Model for studying biofilms on implants

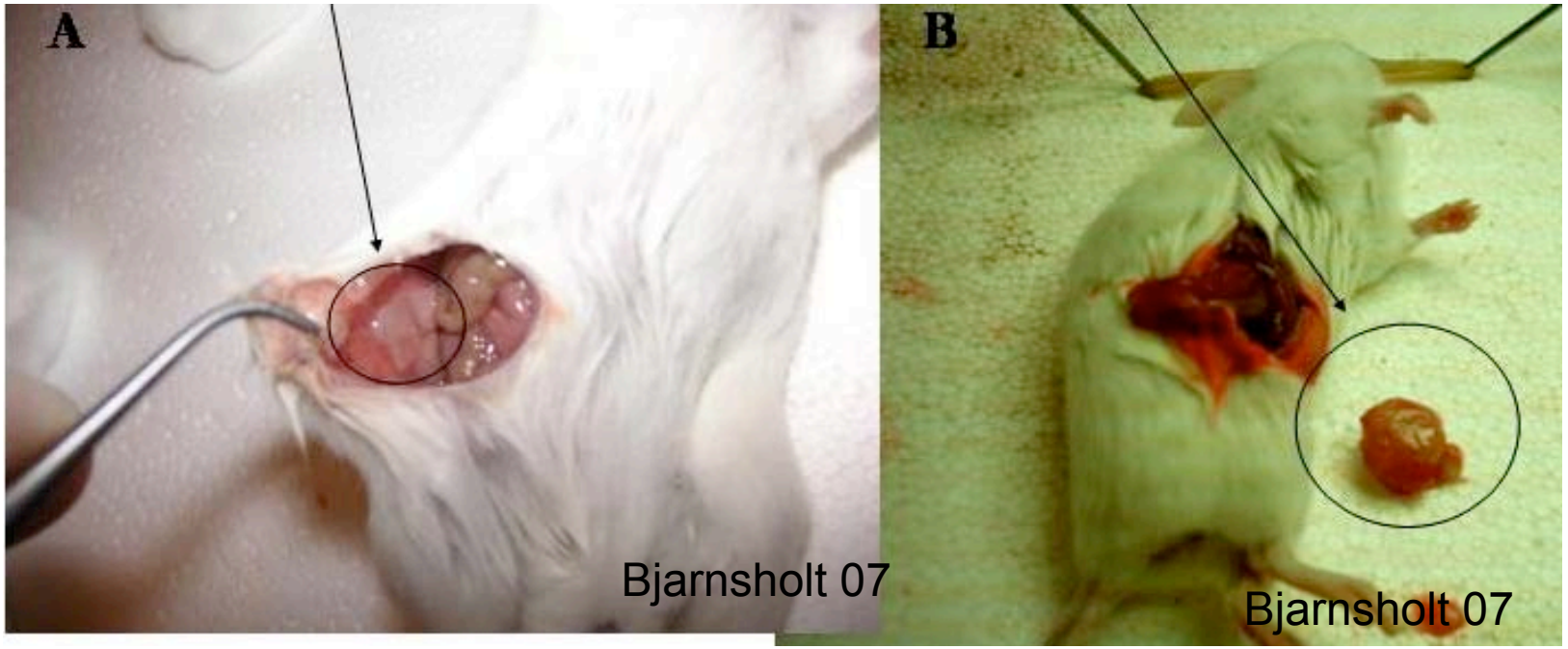
- Aim
  - To introduce an *in vivo* foreign-body infection model, with the aim of later treating the infection
  - The model is calibrated when the mice do not die upon insertion of a *P. aeruginosa* colonized implant and bacteria can be found on the implants 1 week post-surgical

Model for studying biofilms on implants

- Square silicone implants are inserted in the peritoneal cavity of mice
- Implants pre-colonized with bacteria
- O/N culture Dilute to OD_{600} 0.5 in 0.9% NaCl. Allow colonization for 20 hours with moderate shaking. Wash the implants before usage.
- Implants removed after different periods of time and studied using CLSM and CFU counting



Abscesses surrounds the wild-type colonized implants in NMRI mice 15 days post-surgical



Application

- The impact of *Pseudomonas aeruginosa* Quorum Sensing (bacterial cell to cell communication) on biofilm persistence on implants *in vivo*

Background

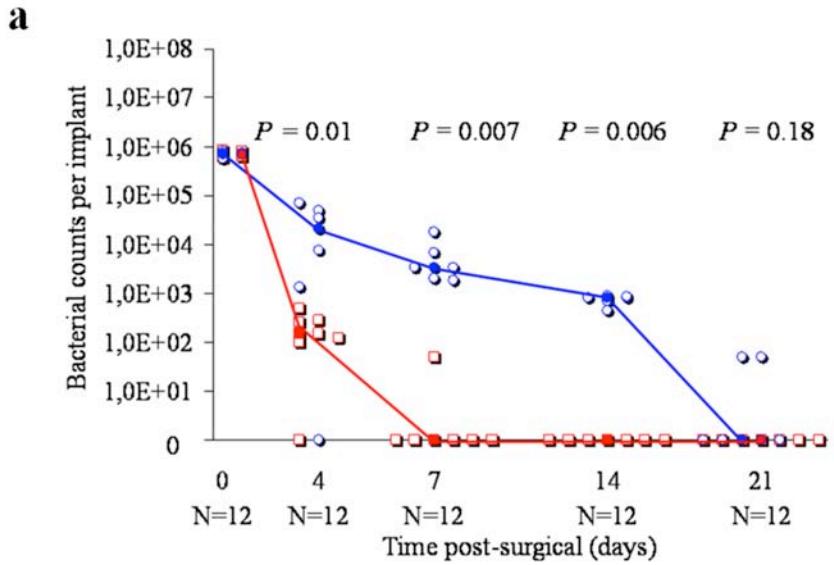
P. aeruginosa QS deficiency decreases:

in vitro biofilm tolerance to antibiotics including kanamycin, tobramycin, ciprofloxacin, ceftazidime

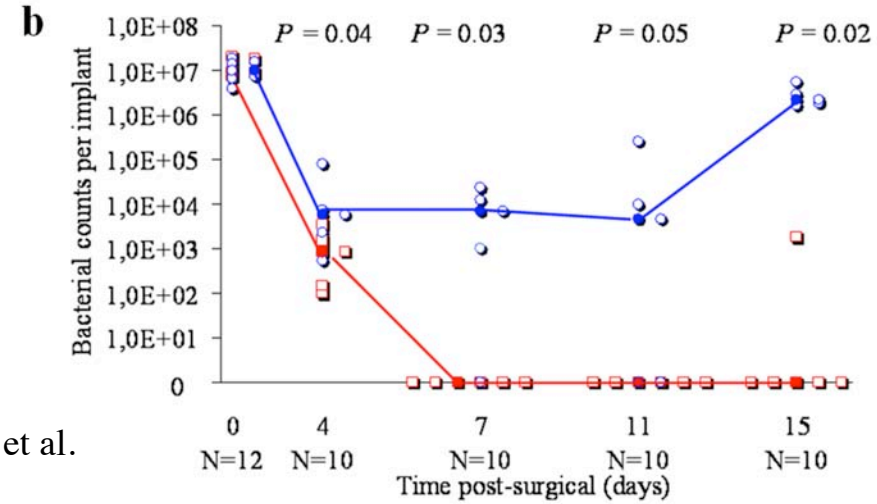
and the tolerance towards the host defense.

Clearing of *P. aeruginosa* wild-type vs. the *ΔlasR rhlR* mutant

Balb/c mice



NMRI mice



Red: *ΔlasR rhlR*

Blue: Wild type

Louise Dahl Christensen et al.
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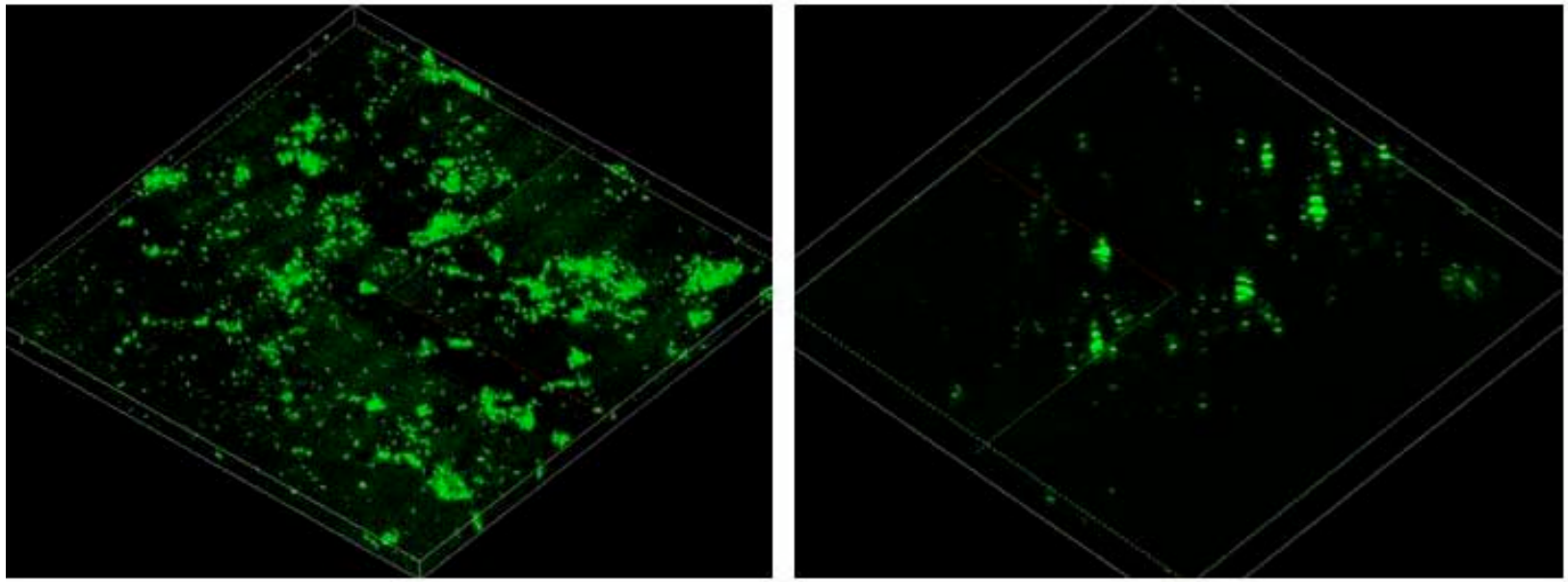
CLSM images: Wild-type vs. the QS mutant

Wild-type *ΔlasR rhlR*



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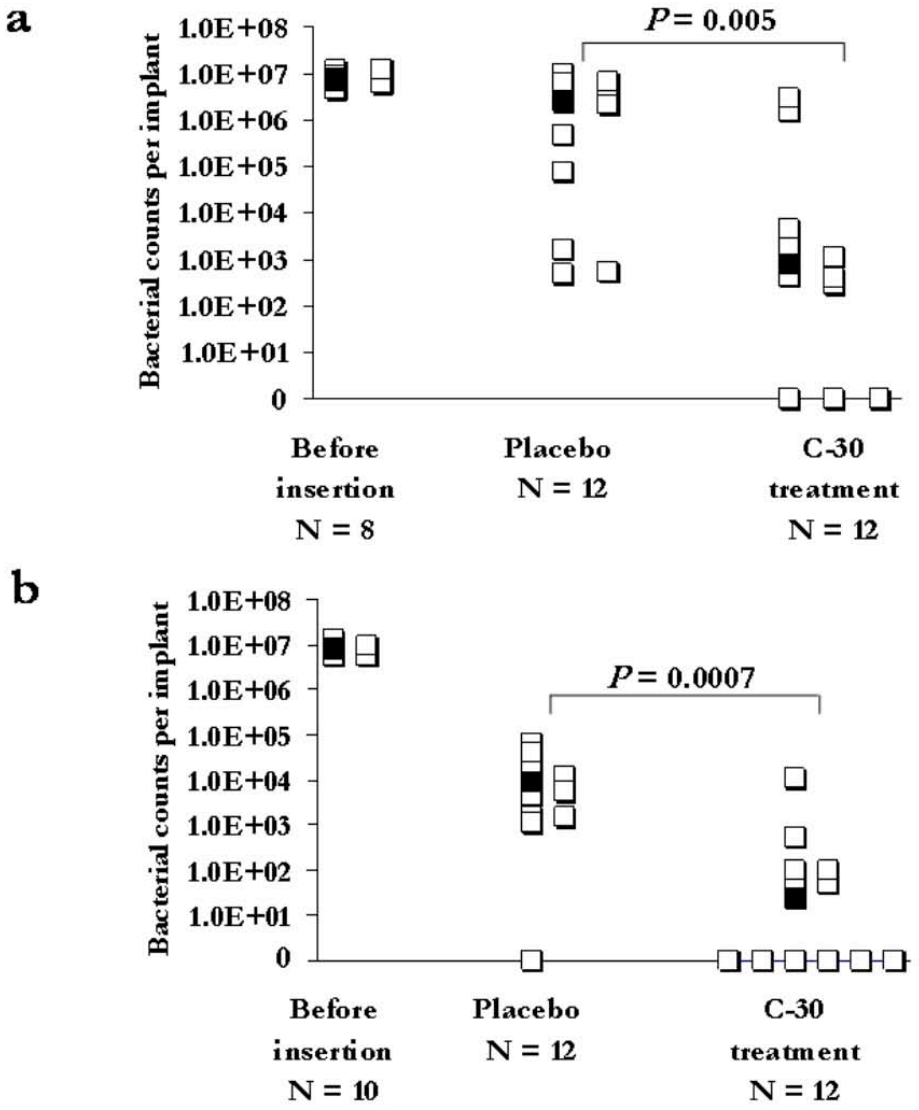
Treatment of wild-type *P. aeruginosa* with the QSI furanone C-30

- It has earlier been shown that mice with wild-type *P. aeruginosa* lung infections cleared the infection significantly faster when treated with furanone C-30 as compared to the control group (Hentzer *et al.*, 2003)

- <u>Hypothesis:</u>

  Treatment with furanone C-30 is able to facilitate clearing of wild-type *P. aeruginosa* colonizing silicone implants placed in the peritoneal cavity of mice, by blocking QS.

C-30 treatment



Louise Dahl Christensen et al.
Microbiology 2007

Conclusion I

- We have successfully established a model to investigate implant related infections
- The model confirmed that the wild-type *P. aeruginosa* is more virulent than the *ΔlasR rhlR* mutant
- QS plays a role in the ability of mice to clear a *P. aeruginosa* foreign-body infection

Conclusion II

- Furanone C-30 is facilitating the mice's ability to clear the wild-type *P. aeruginosa* from silicone implants
- The treatment effect of furanone C-30 is independent of the mouse strain
- This *in vivo* model will be an important tool in the testing of novel antimicrobials against *P. aeruginosa* biofilm infections

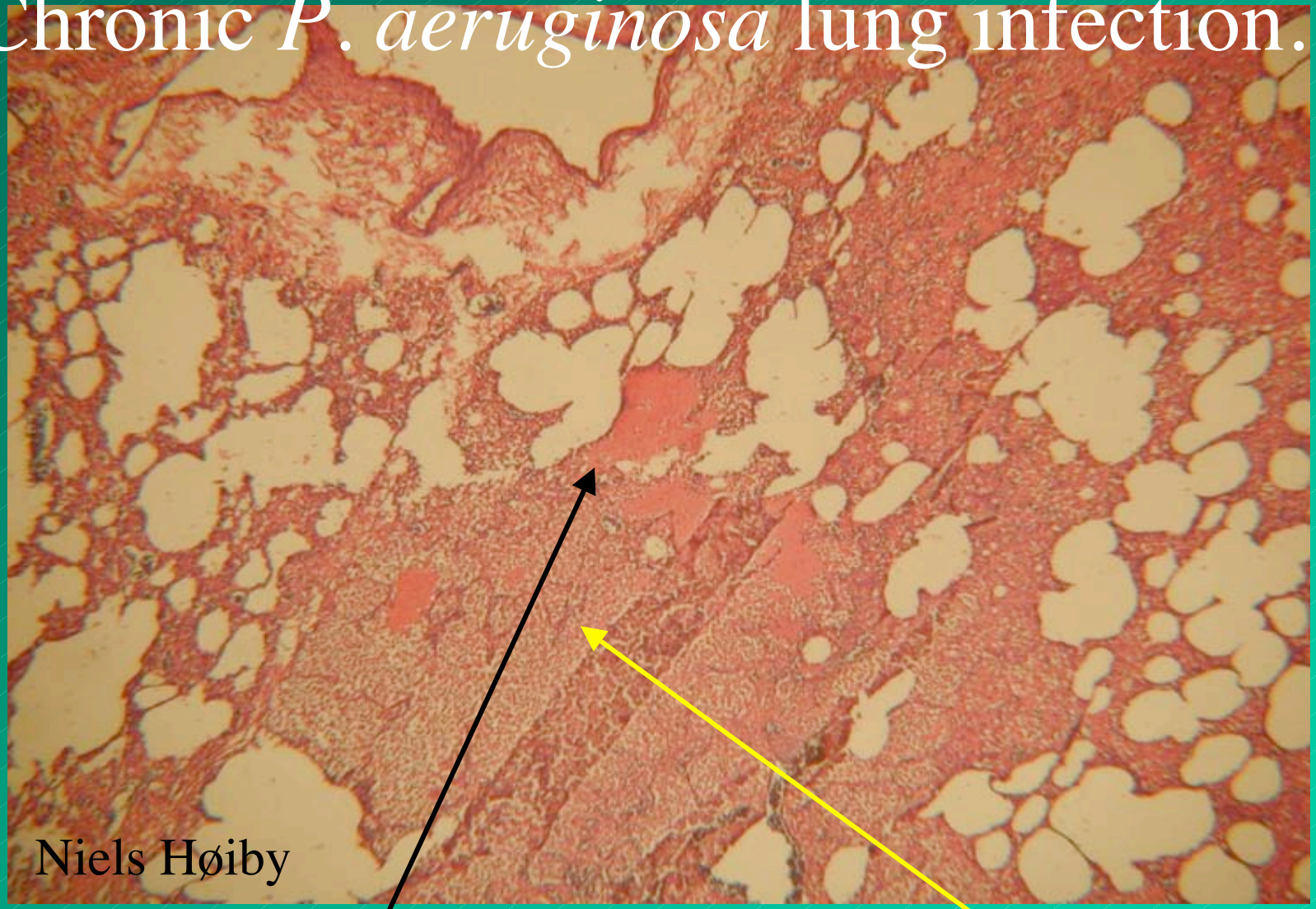
Chronic *Pseudomonas aeruginosa* lung infection models

2009

Cystic fibrosis

- Most common lethal, somatic, recessive inherited disease. Approximately 30-40,000 (EU) and 20,000 (USA) patients.
- Caused by mutations in the cystic fibrosis transmembrane conductance regulator gene (CFTR), a Chloride channel.
- Results in dehydrated airway surface liquid and subsequently impairment of the mucociliary escalator.
- Characterized by recurrent lung infections in childhood.

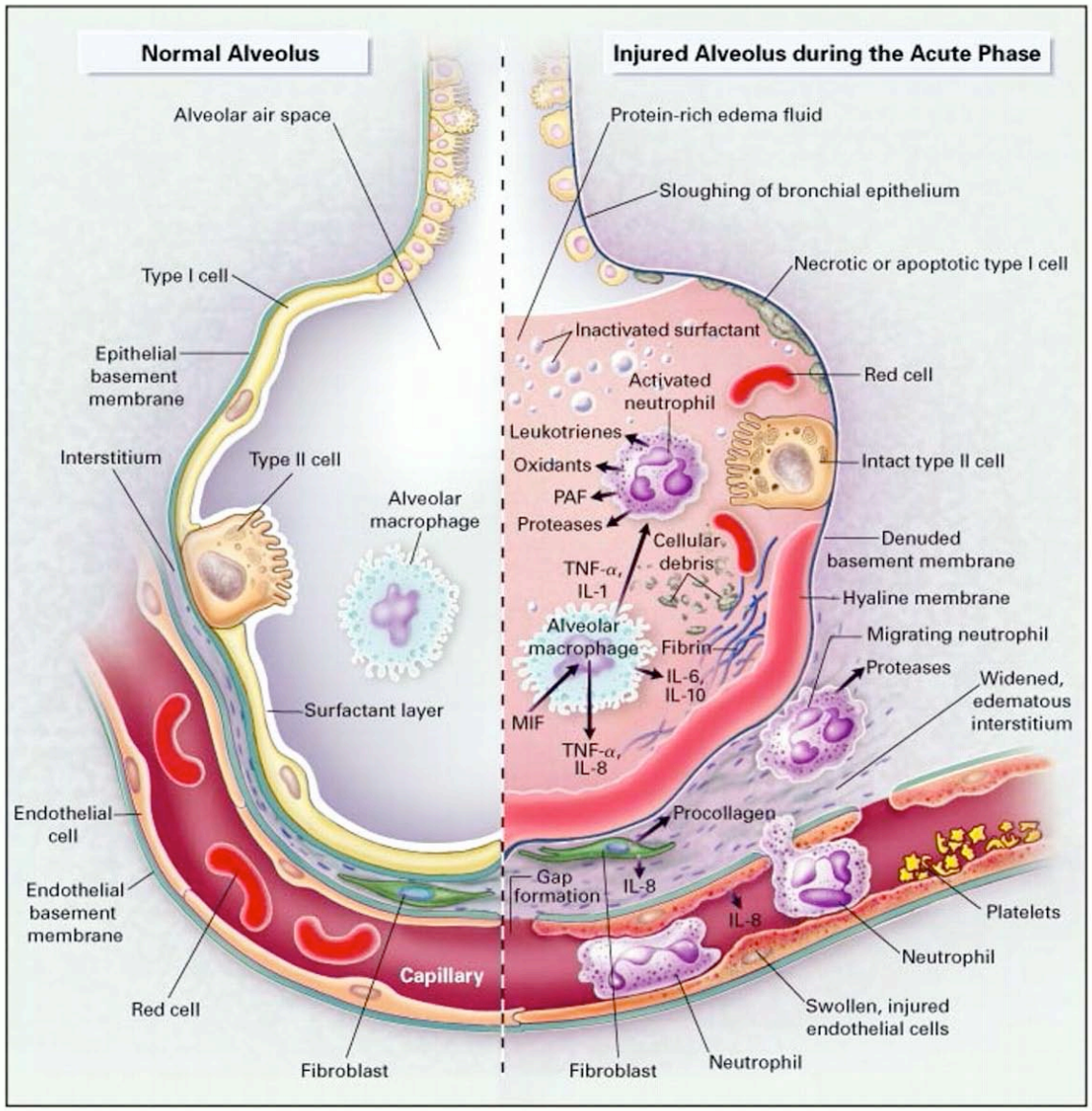
Chronic *P. aeruginosa* lung infection.



Mucoid biofilm of P. aeruginosa in an alveolar surrounded by severely inflammed tissue (PMNs, pneumonia). Autopsy (BS242/74) of a CF girl (MLM) who died due to chronic P. aeruginosa lung infection and 21 precipitating antibodies against P. aeruginosa. HE stain x 40

Courtesy of Niels Høiby

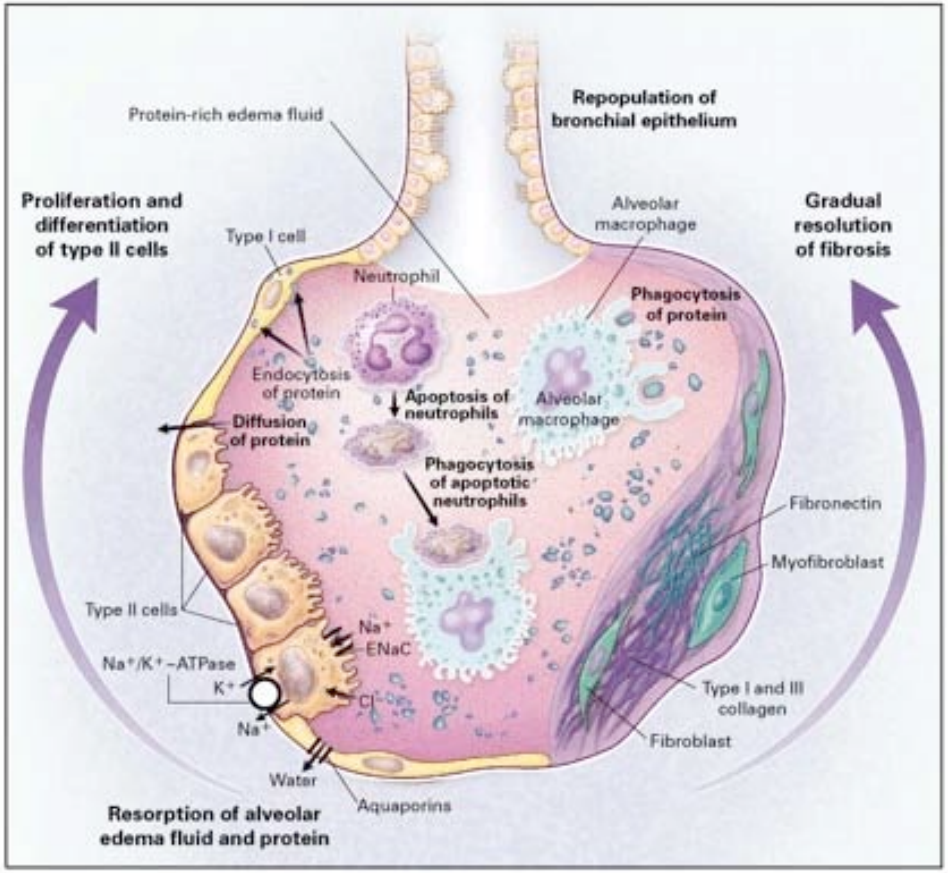
Lung inflammation



Ware LB, Matthay MA. NEJM 2000

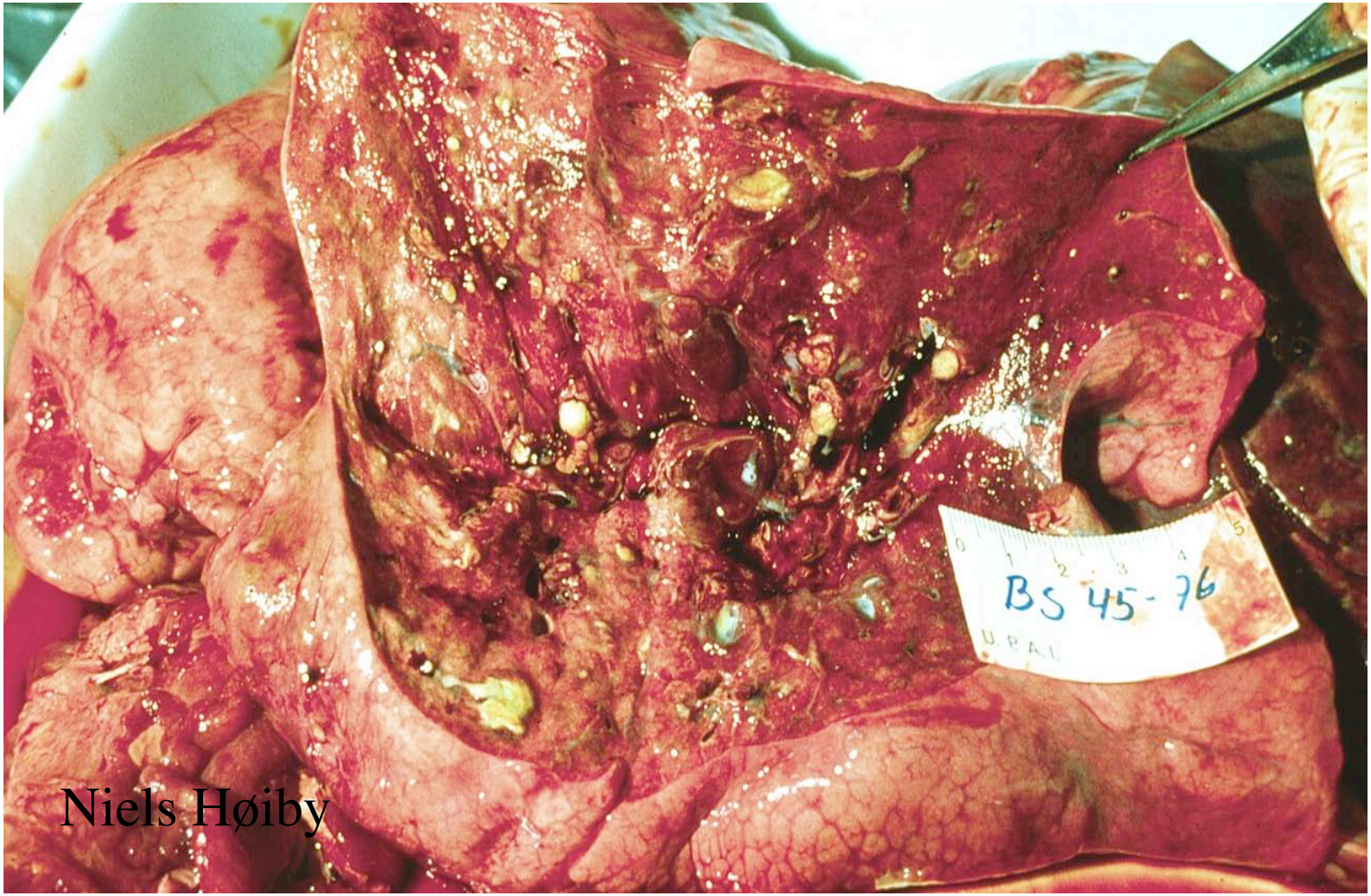
- In addition:
  - Cytokines: IL-6, G-CSF, GM-CSF, IL-3, PGE2, LTB4, IL-10
  - Cells: Dendritic cells, mast cells, NK-cells, endothelial cells, fibroblasts, thrombocytes

Lung resolution



Ware LB, Matthay MA. NEJM 2000

- **Inflammation is accelerated upon activation of adaptive immune response!**
  - **Skewing of Th1/Th2 balance**
  - **Immunecomplex disease**
  - **Paradox persistent acute type inflammation (PMNs)**
  - **Progressive loss of lung function**
  - **Fibrosis**



Autopsi of Cystic Fibrosis lung with chronic *Pseudomonas aeruginosa* infection.

Courtesy of Niels Høiby

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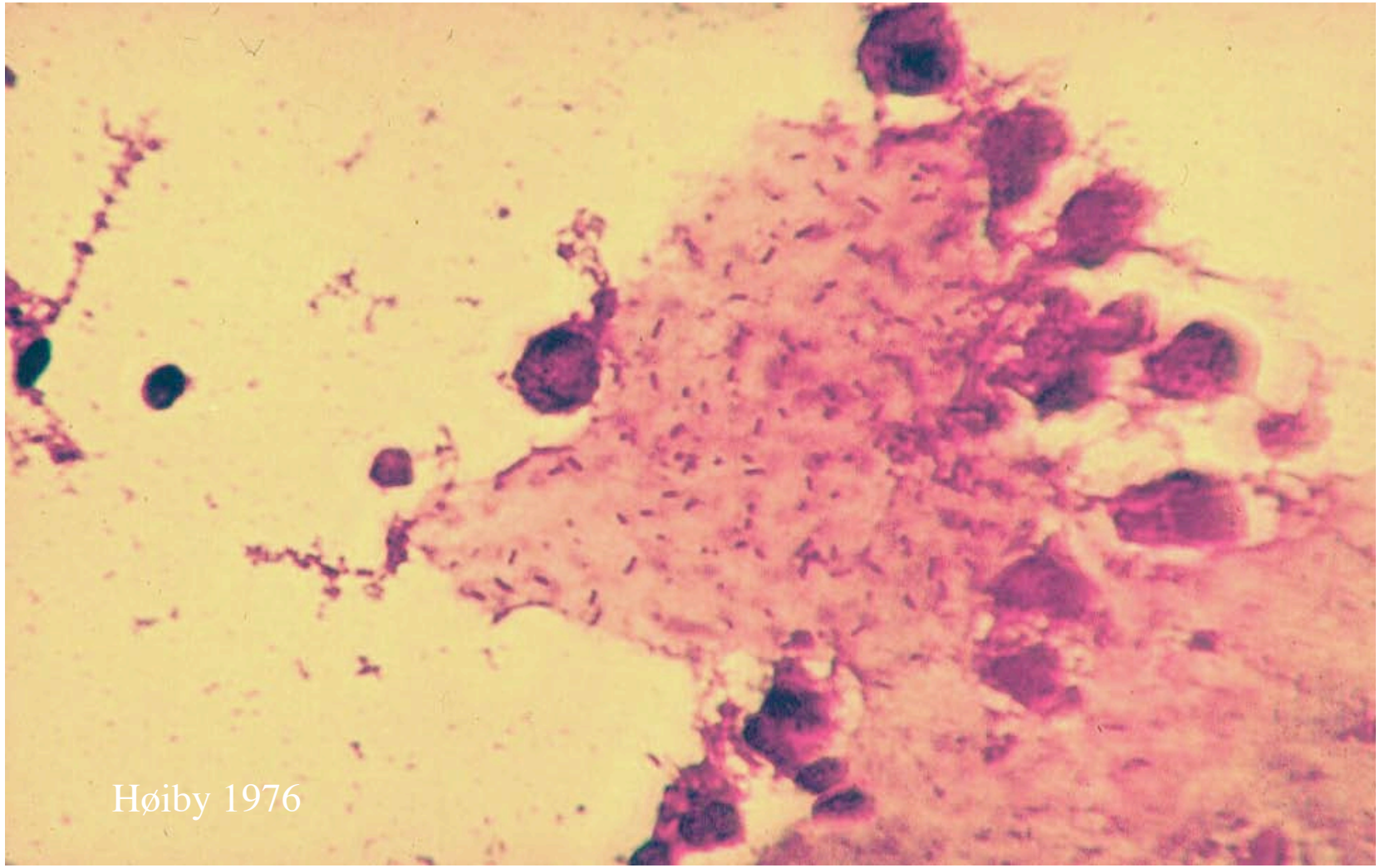
Major characteristics of chronic *P. aeruginosa* lung infection in CF.

- **Biofilm** mode of growth.
- **Mucoid** phenotypes.
- **Tolerance** (in vitro susceptible, but clinical resistant) and increasing **resistance** (in vitro resistant) to antibiotic treatment.

=> Continuous immune responses and tissue destruction.

Biofilm mode of growth.



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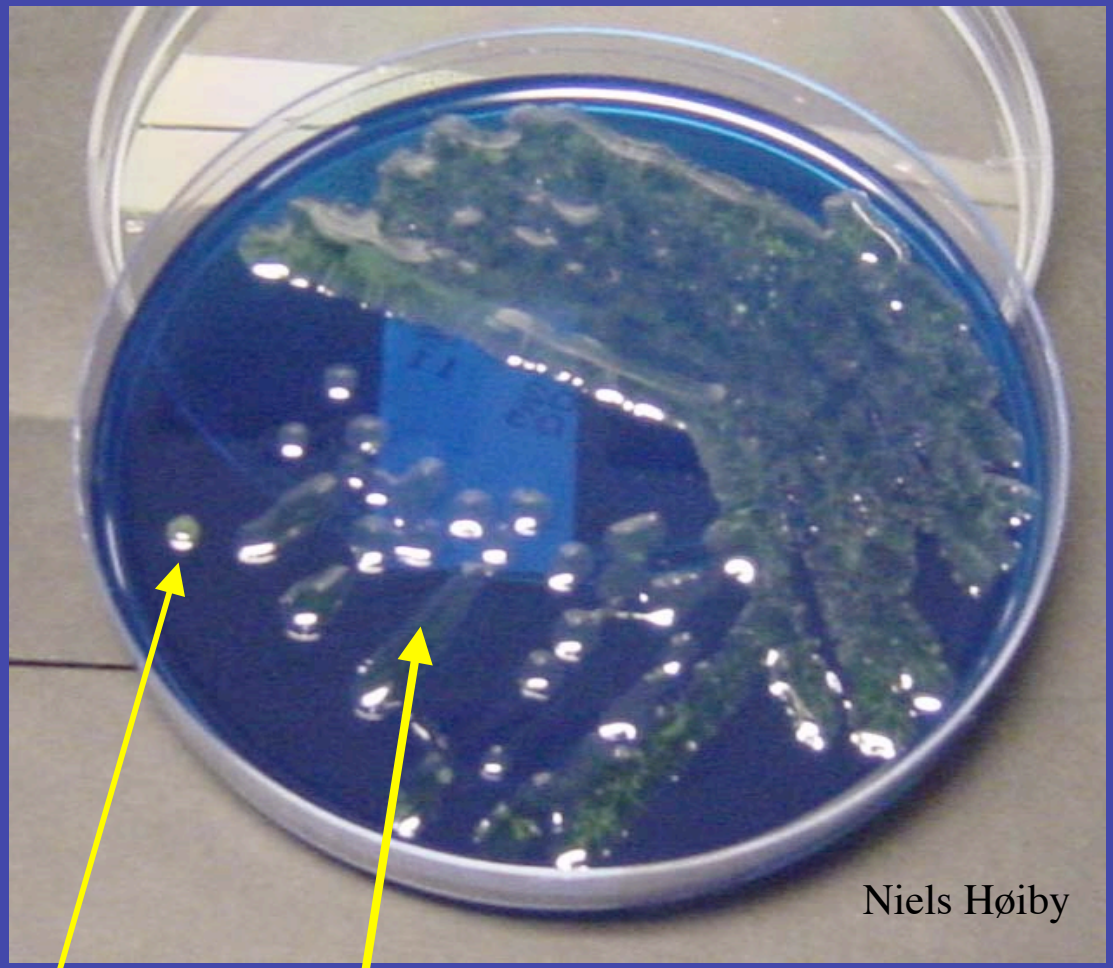
Mucoid phenotypes.



Non-mucoid colony

Mucoid colony

P. aeruginosa from BAL of a cystic fibrosis patient (LFS): mucoid and non-mucoid colonies, hereditary phenotypes, but same genotype:

Høiby.

Increased tolerance to antibiotics

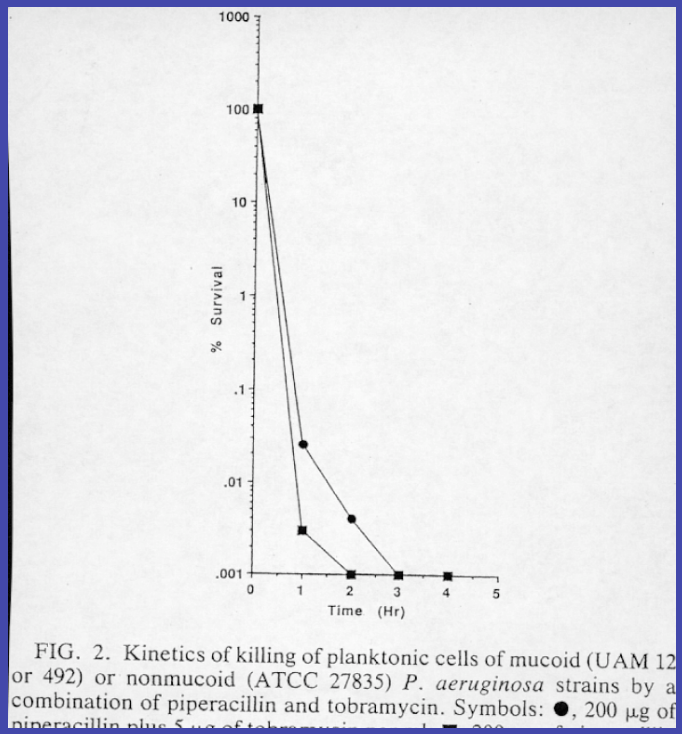


FIG. 2. Kinetics of killing of planktonic cells of mucoid (UAM 12 or 492) or nonmucoid (ATCC 27835) *P. aeruginosa* strains by a combination of piperacillin and tobramycin. Symbols: ●, 200 μg of

Planktonic P. aeruginosa killed by tobramycin and piperacillin

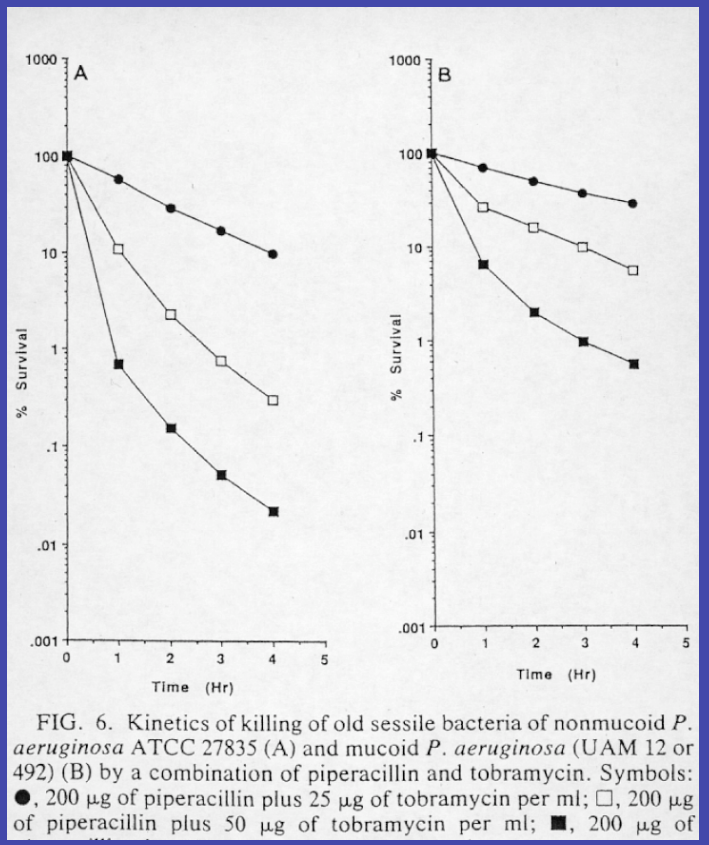


FIG. 6. Kinetics of killing of old sessile bacteria of nonmucoid *P. aeruginosa* ATCC 27835 (A) and mucoid *P. aeruginosa* (UAM 12 or 492) (B) by a combination of piperacillin and tobramycin. Symbols: ●, 200 μg of piperacillin plus 25 μg of tobramycin per ml; □, 200 μg of piperacillin plus 50 μg of tobramycin per ml; ■, 200 μg of

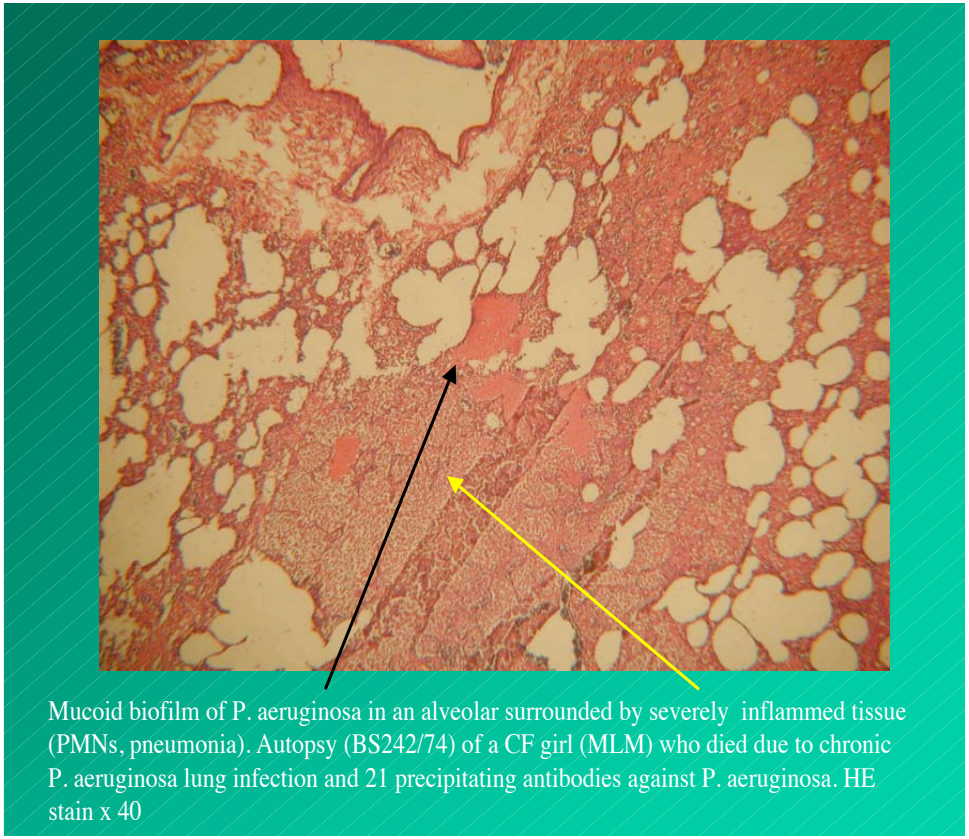
Old biofilm of P. aeruginosa, left Non-mucoid, right: Mucoid survives tobramycin and piperacillin

Anwar & Costerton: Enhanced activity of combination of tobramycin and piperacillin for eradication of sessile biofilm cells of P. aeruginosa. AAC 1990;34:1666-71

Høiby, N

Cystic fibrosis. Multiorgan disease

- Malabsorption, pancreatic insufficiency
- Male infertility
- Hepatic insufficiency
- Diabetes mellitus
- Allergy
- Kidney insufficiency
- **Chronic endobronchial infection**



Mucoid biofilm of P. aeruginosa in an alveolar surrounded by severely inflammed tissue (PMNs, pneumonia). Autopsy (BS242/74) of a CF girl (MLM) who died due to chronic P. aeruginosa lung infection and 21 precipitating antibodies against P. aeruginosa. HE stain x 40

Mouse models

Pro's

- Numerous inbred strains
- Prolonged infection possible
- Inflammation CF-like
- Vast immunological tools available
- CF mice available (and ENaC mice)

Con's

- Important histological differences
- Natural resistant to *P. aeruginosa*
- (No spontaneous lung infection in CF mice)
- Short lifespan

Dominated by rat and mouse models

The lung infection models

- Embedded models (”Classical”)
  - Agar (Cash 1979)
  - Agarose
  - Seaweed alginate
  - Mucoid model in native alginate (”de Novo”)
  - PFGE-identical strains isolated from one patient over years
- Planktonic models
  - Nebulizing chamber
  - Nasal application/influtation
  - Drinking model
- Tube model (”Japanese”)
- Xenograft model

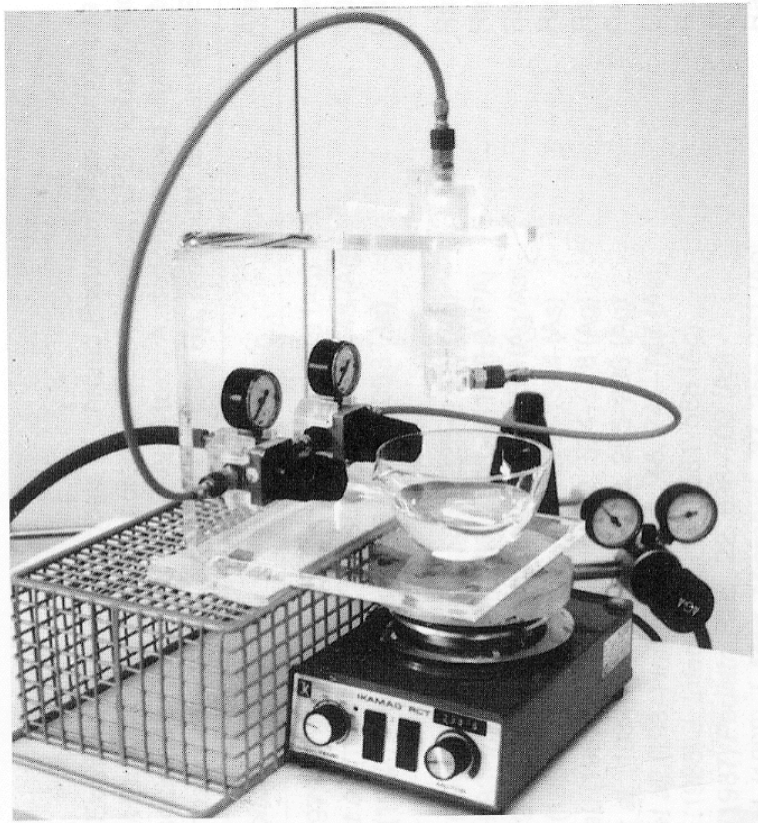


Figure 61.3 The set-up for alginate bead preparation, showing the cylindrical reservoir where the bacterial suspension is placed as well as the mounting of the tubes for air. The $CaCl_2$–TRIS–HCl buffer is placed on a magnet-stirrer.

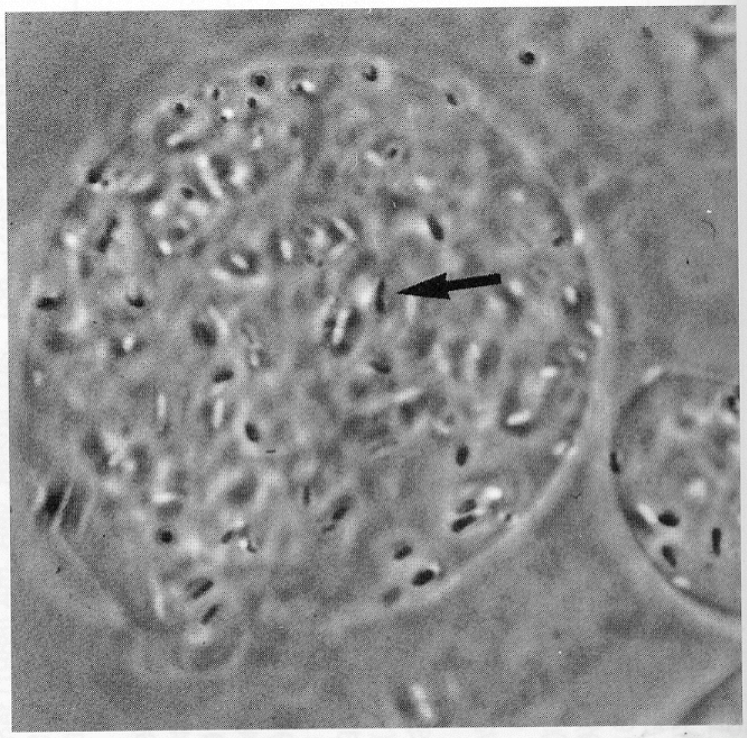


Figure 61.4 Phase contrast microphotograph of an alginate bead containing numerous *Pseudomonas aeruginosa* bacteria (arrow). Magnification × 1000. (From Johansen *et al.*, 1993, reproduced with permission from the editor of APMIS).

Seaweed alginate beads 60 μm (30-110 μm)

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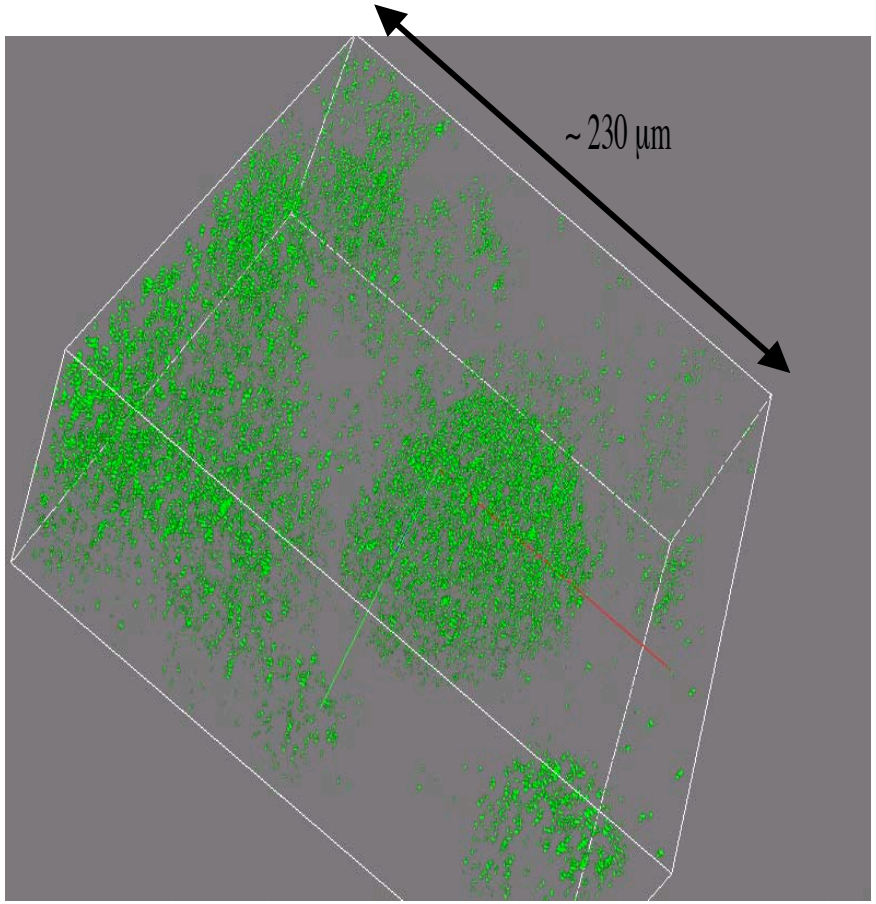
Confocal microscopy picture of alginate beads.



Courtesy Thomas Bjarnsholt.

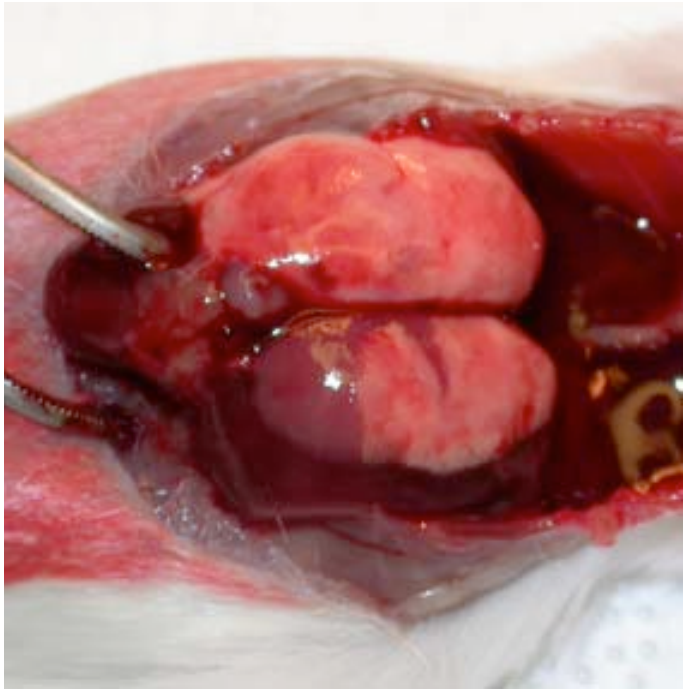
Characteristics.

The seaweed alginate model (Pedersen SS, et al. APMIS 1990)

- A clinical alginate producing isolate PAO 579 (provided by Govan, Edinburgh). 10^{7-8} CFU/ml, 0.1 ml/rat.
- Intratracheal infection with beadtipped needle
- Sacrifized rats 4 weeks after challenge.
- Compared to the agar model.
- Macroscopic pathology:
  - Grey nodules (abscesses) and pleural adhesions.
- Histological pathology:
  - Pronounced PMN dominated inflammatory response. Beads with bacteria.
- Quantitative bacteriology:
  - Positive bacteriology in 10/12 rats.
- Antibody production:
  - Significantly higher number of precipitating antibodies in the alginate group.

Characteristics mice (Using PAO 579)

- Course of infection highly dependent on mouse strain.
- Shorter duration of the infection (1-2 weeks).
- Macroscopic abscesses are seldom.



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Characteristics mice.

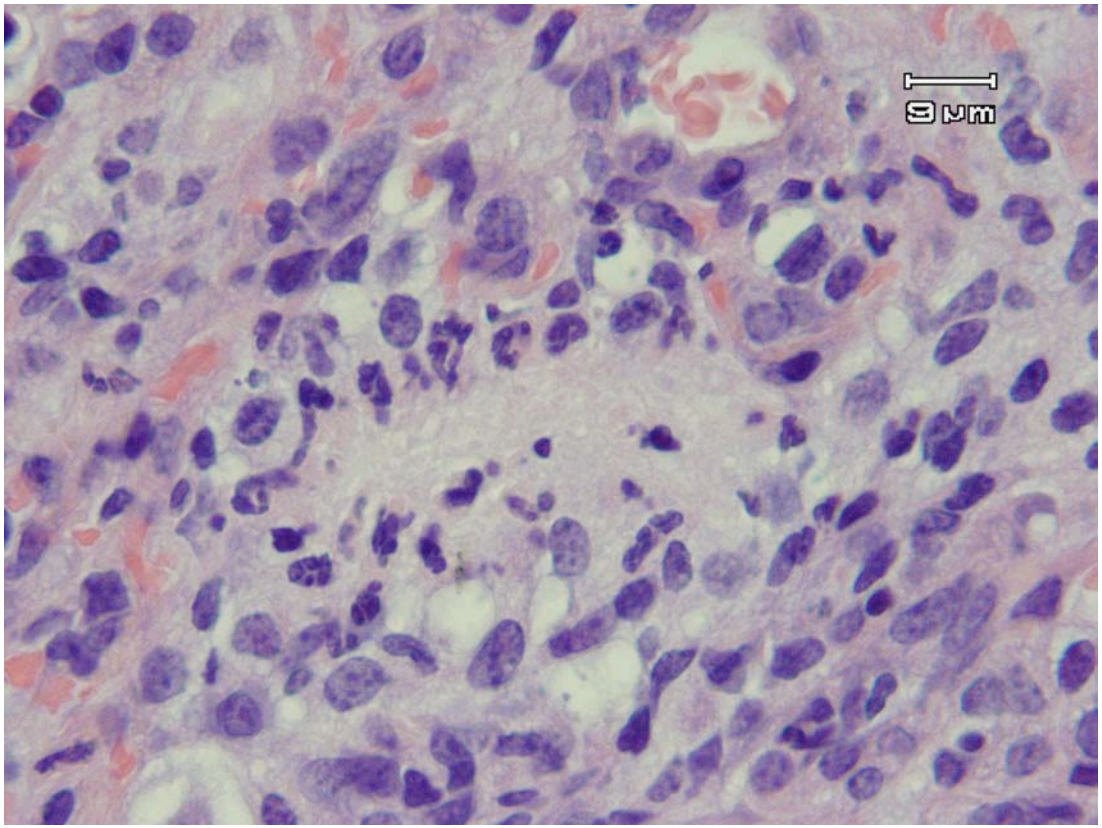
(Using PAO 579)

- Course of infection highly dependent on mouse strain.
- Shorter duration of the infection (1-2 weeks).
- Macroscopic abscesses are seldom.
- Similar histopathology.

Histopathological evaluation

- By a pathologist
  - x2-400, 5-10 fields
- Type
  - Acute type (PMN dominated)
  - Chronic type (MN dominated)
  - PMN/MN type
- Granulomas, microabscesses, athelectasis etc.
- Degree
  - 0 (no inflammation) -> +++ (heavy inflammation)
- Evaluated blindly
- Semiquantitative
- Reproducible

Histopathology



- Severe inflammation
- Both central and in the periphery
- Alginate area with biofilm like structures

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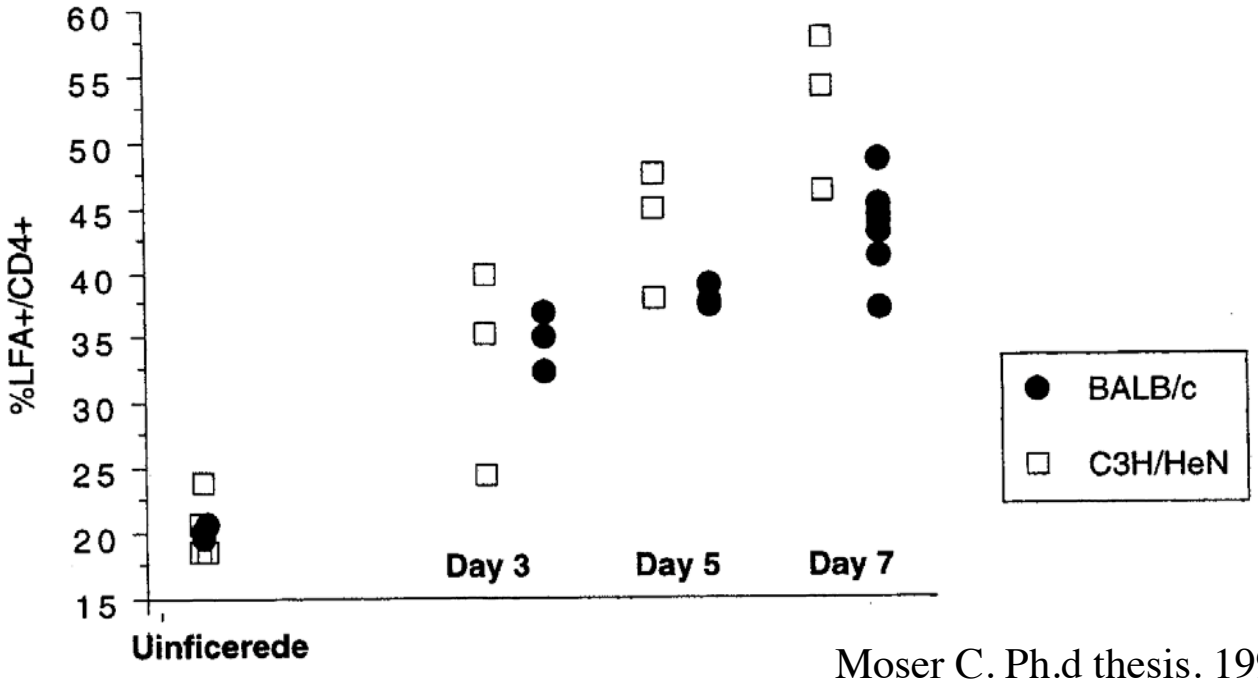
Characteristics mice.

(Using PAO 579)

- Course of infection highly dependent on mouse strain.
- Shorter duration of the infection (1-2 weeks).
- Macroscopic abscesses are seldom.
- Similar histopathology.
- Antibody production, and activation of cellular immunity.

Activation of CD4+ cells



Moser C. Ph.d thesis. 1999.

Use of the model

- Vaccination studies (HK Johansen, O Ciofu)
- Immune modulation (C Moser, HK Johansen, PØ Jensen)
- Antibiotic resistance (O Ciofu)
- Immune responses (C Moser, PØ Jensen)
- Treatment studies (C Moser, Z Song, T Bjarnsholt)
- Host-Pathogen interactions (H Wu, T Bjarnsholt, PØ Jensen, S Prakhabar)

Modifications of the model

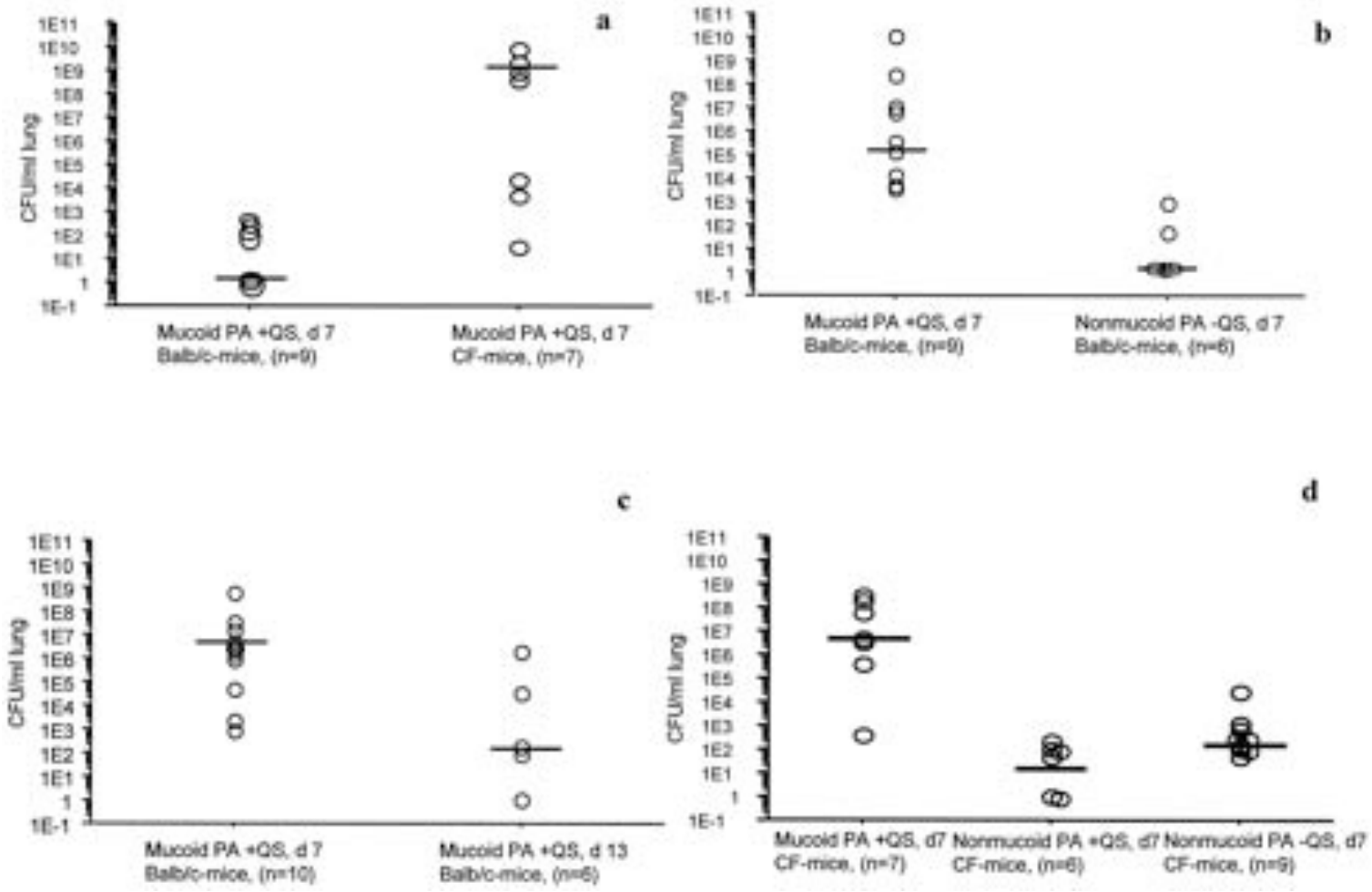
- Re-infection
- Embedment in native alginate
- Adaptation model

Mucoid *P. aeruginosa*

(Hoffmann N, et al. IAI 2005)

- Stabile mucoid phenotype.
- Cultured for 28h, 37°C.
- Centrifuged, and resuspended in 2 ml ox broth.
- Adjusted to $1x10^8$ CFU/ml ($1x10^9$ for BALB/c mice) in crude or purified native alginate.
  - Crude: culture supernatant
  - Purified: supernatant heated to 80°C for 30 min. Precipitated with 99% ice-cold ethanol. Resuspended in sterile 0.9% saline.
- Mice challenged intra-tracheally with a bead-tipped needle.
  - CF-mouse: *cftr*^{tm1Unc}-TgN$^{(FABPCFTR)}$ (Jackson Lab.)
  - BALB/c mouse (M&B Lab.)

Quantitative bacteriology



Mucoid *P. aeruginosa* embedded in crude native alginate induced higher mortality of lung infected BALB/c mice as compared to the non-mucoid isolate ($p<0.05$).

Hoffmann N, et al. IAI 2005.

Macroscopic pathology

Return to article

TABLE 3. Macroscopic lung pathology after intratracheal *P. aeruginosa* challenge of CF mice and BALB/c mice with the mucoid strain +QS (NH57388A), the nonmucoid strain –QS (NH57388B), and the nonmucoid strain +QS (NH57388C)[a]

	Pathology (no. of mice in scoring groups/total no. of mice challenged [%]) at day 7								
	Expt 1		Expt 2		Expt 3		Expt 4		
Score[c]	CF mice (mucoid +QS*** NH57388A)	BALB/c mice (mucoid +QS NH57388A)	BALB/c mice (mucoid +QS**** NH57388A)	BALB/c mice (nonmucoid –QS NH57388B)	BALB/c mice (mucoid +QS* NH57388A)	BALB/c mice (mucoid + QS NH57388A)[b]	CF mice (mucoid +QS NH57388A)	CF mice (nonmucoid +QS** NH57388C)	CF mice (nonmucoid –QS***** NH57388B)
1		1/15 (7)		5/12 (42)					1/9 (11)
2		10/15 (67)		7/12 (58)		2/6 (33)	1/7 (14)	6/6 (100)	8/9 (89)
3	2/9 (22)	3/15 (20)	4/10 (40)		8/10 (80)	2/6 (33)	2/7 (29)		
4	7/9 (78)	1/15 (7)	6/10 (60)		2/10 (20)	2/6 (33)	4/7 (57)		

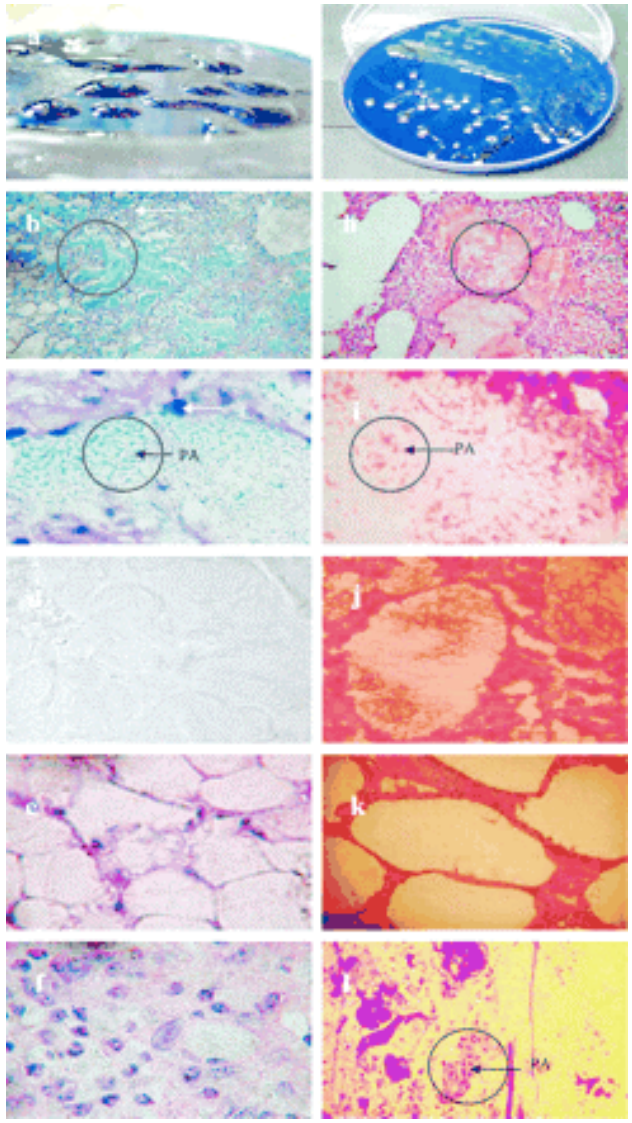
[a] See Table 2, footnote *a*. Asterisks indicate probabilities as follows: *, $P < 0.01$ compared to day 13; **, $P < 0.01$ compared to the mucoid strain NH57388A +QS; ***, $P < 0.001$ compared to BALB/c mice; ****, $P < 0.001$ compared to the nonmucoid strain NH57388B –QS; *****, $P < 0.001$ compared to the mucoid strain NH57388A +QS.

[b] Day 13, in this case.

[c] Score 1, normal; 2, swollen lungs, hyperemia, small atelectasis; 3, pleural adhesion, atelectasis, multiple small abscesses; 4, large abscesses, large atelectasis, and hemorrhages.

Hoffmann N, et al. IAI 2005.

Histopathology



x40

Microscopy of CF-mouse lung (HE and Alcian blue)

x100

CF-patient

Mucoid in CF-mouse

Confocal microscopy of freeze microtomy sections of mouse lungs

Saline

Alveoles without infection

Non-mucoid BALB/c-mouse

CF-sputum

Hoffmann N, et al. IAI 2005.

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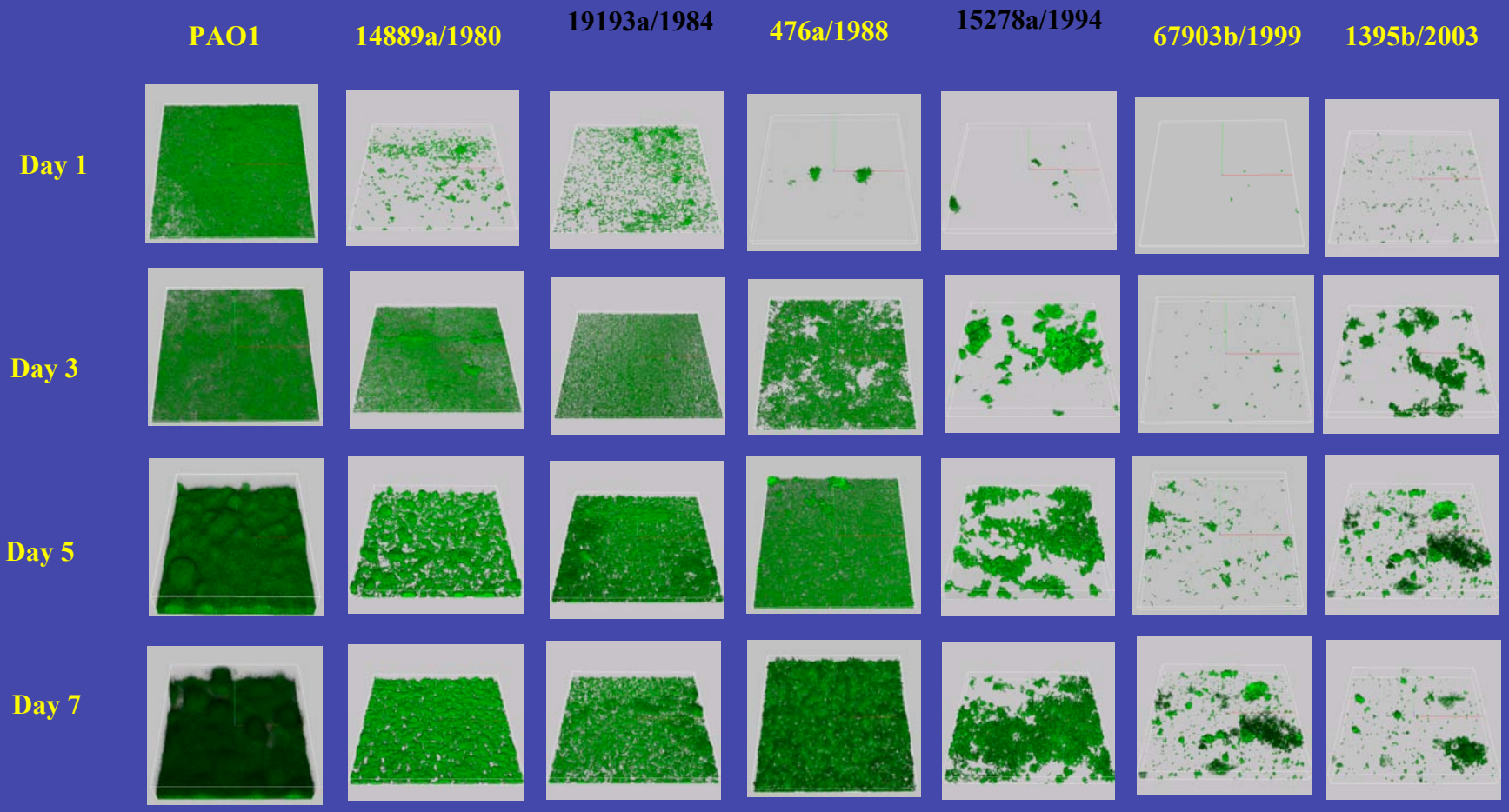
Cystic fibrosis - the chronic infection.

- 20-30 years of chronic infection.
- ≥ 10,000 days (~ 120,000 bacterial generations) of mutual exposure to the inflammatory responses and bacterial virulence factors.
- ≥ 100 antibiotic i.v. courses (≥ 1,400 days) of antibiotic exposure.
- Mouse models of chronic *P. aeruginosa* lung infections are limited to 2 (- 3) weeks!

CLSM images of biofilm formed by longitudinal *P. aeruginosa* isolates



Biofilm development of wild type PAO1 and sequential isolates from patient no 1. Flow chambers were inoculated with *gfp*-tagged wild type and *P. aeruginosa* isolates grown on Casamino Acids minimal media. CLSM images were acquired at 1, 3, 5 and 7 days after inoculation.

Baoleri Lee, et al. J Clin Microbiol 2005

Phenotypic analysis of non-mucoid *Pseudomonas* Isolates

Patient no.	Isolates name/year	Chronic infection period (years)	Hypermutability[a]	Colony phenotype[b]	Motilities[c]			Detection of AHL signals[d]		Virulence production[e]	
					Swim	Twitch	Swarm	3-O-C_{12}-HSL	C_4-HSL	Pyocyanin	Proteases
Patient 1	14889a/1980	8	nHp	R	-	+	+	+	+	++	+
	19193a/1984	12	nHp	R	-	+	+	+	+	++	+
	476a/1988	16	nHp	R	-	+	+	+	+	++	+
	15278a/1994	22	Hp	S	-	-	-	-	+	-	-
	67903b/1999	27	Hp	S	-	-	-	-	+	-	-
	1395b/2003	*	Hp	S	-	-	-	-	+	-	-
Patient 2	374d/1985	7	nHp	R	-	+	-	+	+	++	+
	64691c/1999	21	Hp	S	-	-	-	-	+	-	-
Patient 3	1738b/1985	15	nHp	S	+	-	++	+	+	++	+
	54514a/1997	27	Hp	R	++	++	++	+	+	+	+
Patient 4	19696/1984	5	nHp	S	+	-	-	-	-	-	-
	68000d/1999	20	Hp	S	-	-	-	-	-	-	-
Patient 5	21168a/1984	6	nHp	R	-	+	+	+	+	++	+
	15357a/1994	16	nHp	R	-	+	+	+	+	++	+
Patient 6	16020/1991	15	nHp	R	-	+	+	+	+	+	++
	65608a/1999	23	Hp	R	+	+	++	+	+	-	+
Patient 7	20688a/1984	4	nHp	S	+	-	-	-	-	-	-
	5284a/1995	15	HP	S	-	-	-	-	-	-	-
Patient 8	15761/1978	1	nHp	R	++	+	++	+	+	+	++

* Isolate 1395b/2003 was recoverd from 3 years after patient was lung transplanted.
[a]nHp., nonhypermutable, Hp., Hypermutable.
[b]R, rough irregular colony phenotype, S. Smooth regular colony phenotype
[c]++, motility zone ≥ 20mm, +, motility zone ≥ 10mm, -, motility zone ≤ 5mm
[d]Production of AHLs were detected by inspecting the bioluminescence of monitor strain, +, detectable, -, non-detectable
[e]++, high level, +, intermitent level, -, low level

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Baoleri Lee, et al. J Clin Microbiol 2005

Adaptation model

- Established to further investigate the role of adaption and diversity.
- To correlate ability to form *in vitro* biofilm to *in vivo* virulence.
- To introduce a new CF-like time perspective (years) of the chronic lung infection in the animal studies.
- Five groups of BALB/c mice infected with five **non-mucoid** PFGE-identical *P. aeruginosa* clones isolated during 23-years of infection in one CF patient.
- Seaweed alginate embedment of bacteria to delay clearing.

	1980	1988	1997	1999	2003	
Non-mucoid	+	+	+	+	+	Sacrifize day 5

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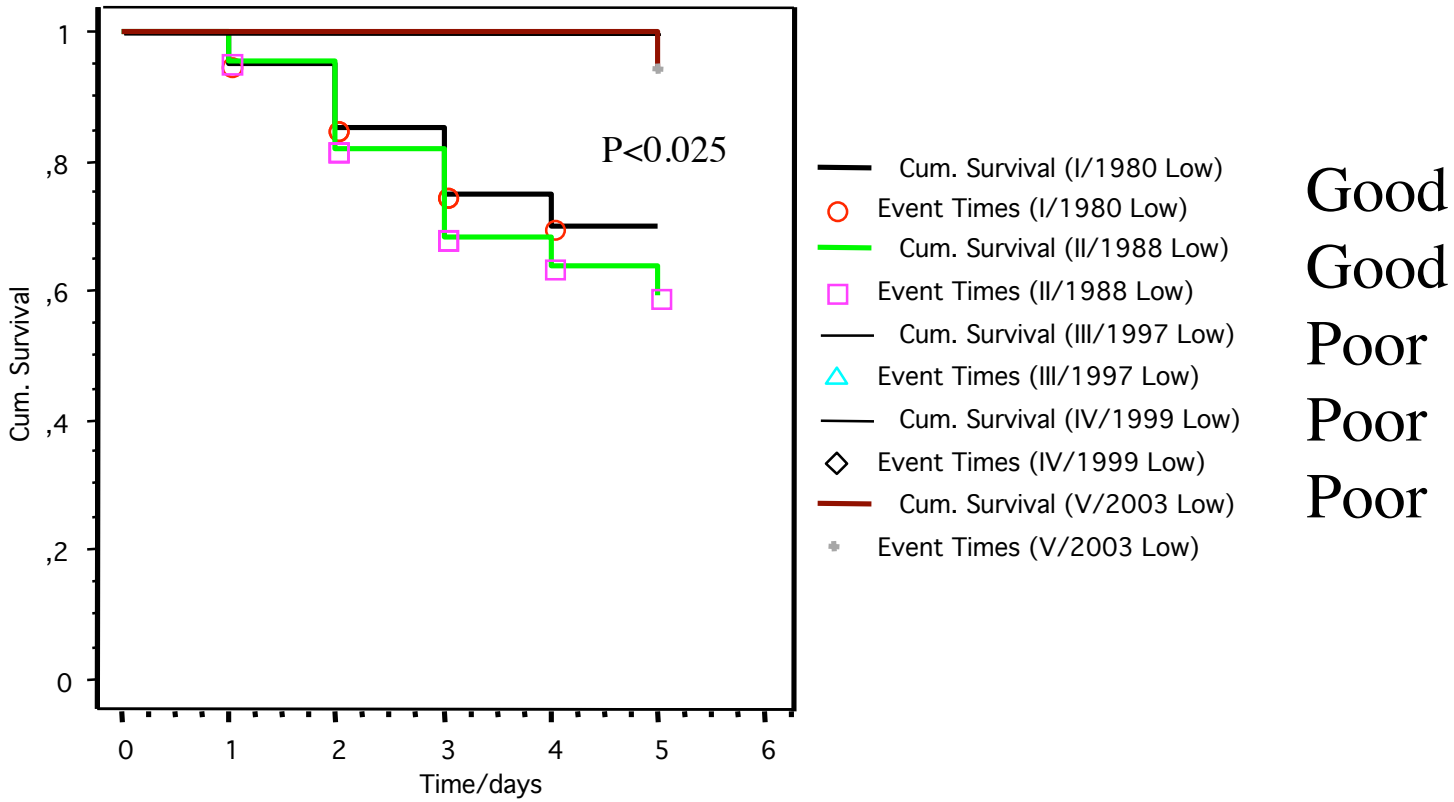
Survival



Good
Good
Poor
Poor
Poor

C Moser *et al*. APMIS 2009

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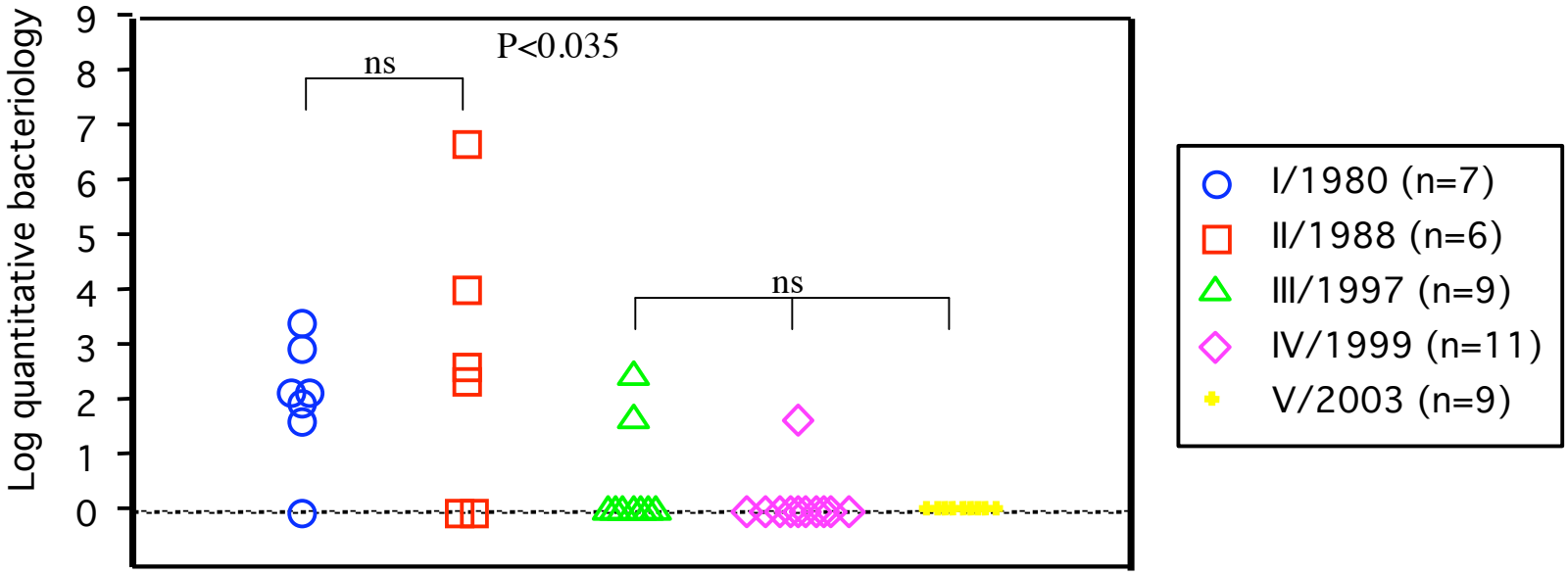
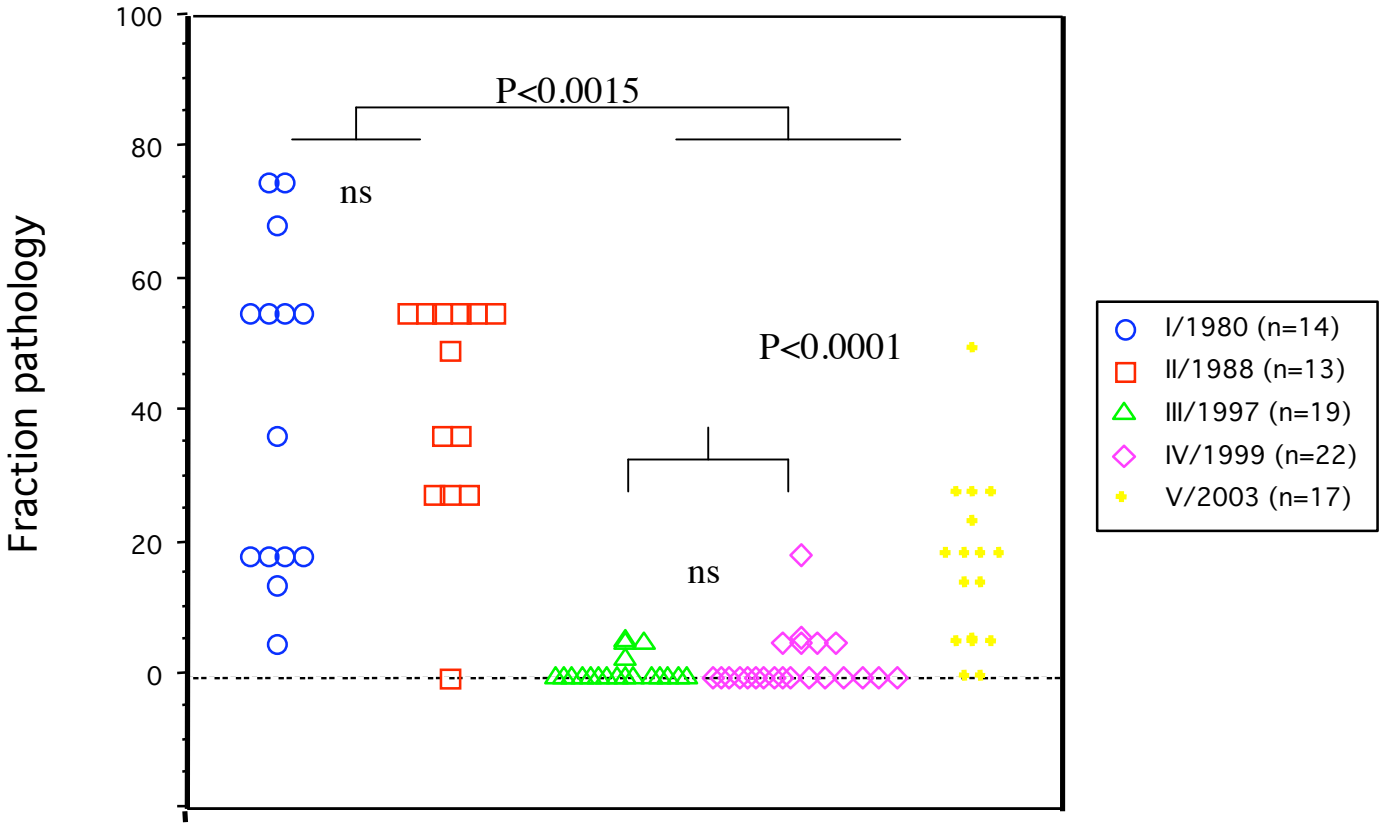
Quantitative bacteriology



C Moser *et al*. APMIS 2009

Claus Moser 2009

Macroscopic pathology



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Histopathology

Groups	Type	Degree	Athelectasis
I/1980 (n=7)	7 PMN/MN* 0 MN 0 NI	4 ++** 3 + 0 -	5 mice§
II/1988 (n=7)	7 PMN/MN* 0 MN 0 NI	2 ++*** 5 + 0 -	4 mice§§
III/1997 (n=10)	2 PMN/MN 0 MN 8 NI	0 ++ 2 + 8 -	No mice
IV/1999 (n=11)	2 PMN/MN 0 MN 9 NI	0 ++ 2 + 9 -	1 mice
V/2003 (n=8)	3 PMN/MN 1 MN 4 NI	1 ++ 3 + 4 -	1 mice

*Significantly more mice with a PMN involving (= acute type) inflammation as compared to group III and IV ($p<0.05$). **Significantly higher degree of inflammation as compared to group III, IV and V ($p<0.02$). ***Significantly higher degree of inflammation as compared to group III and IV ($p\leq0.0002$). §Significantly more mice with athelectasis as compared to group III, IV and V ($p<0.05$). §§Significantly more mice with athelectasis as compared to group IV and V ($p<0.05$).

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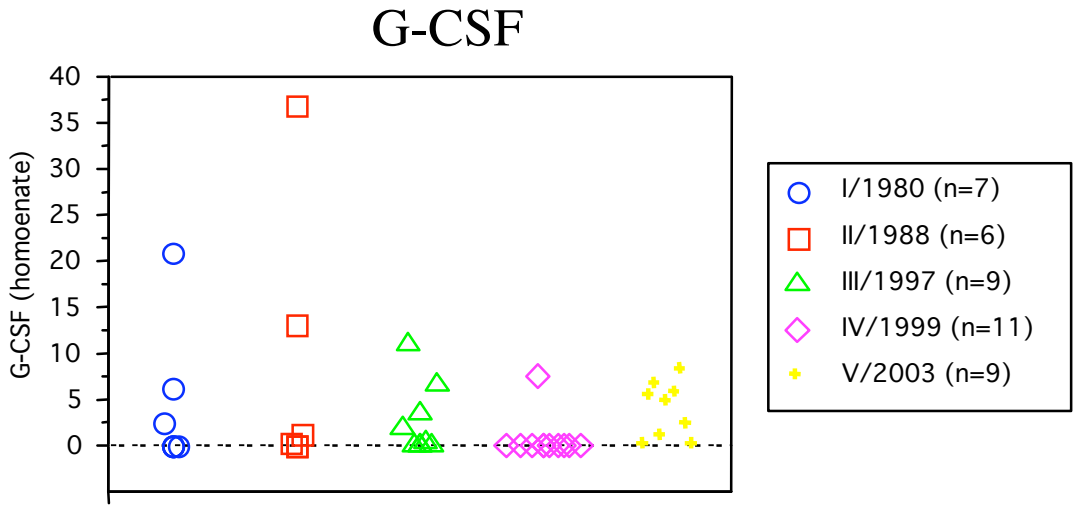
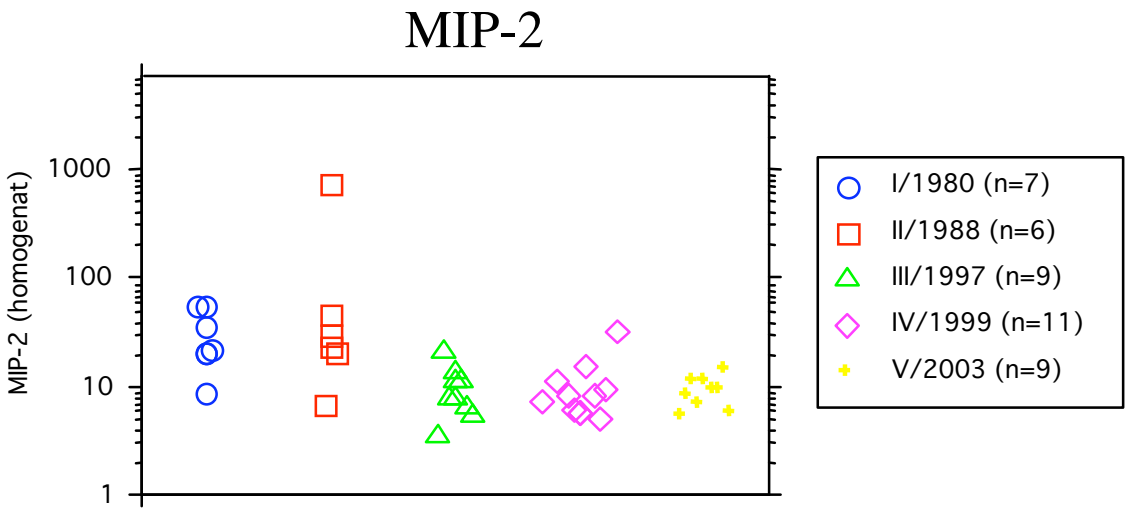
G-CSF and MIP-2 decreases.

PMN mobilizer



Chemoattractant



C Moser *et al*. APMIS 2009

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Partial conclusion

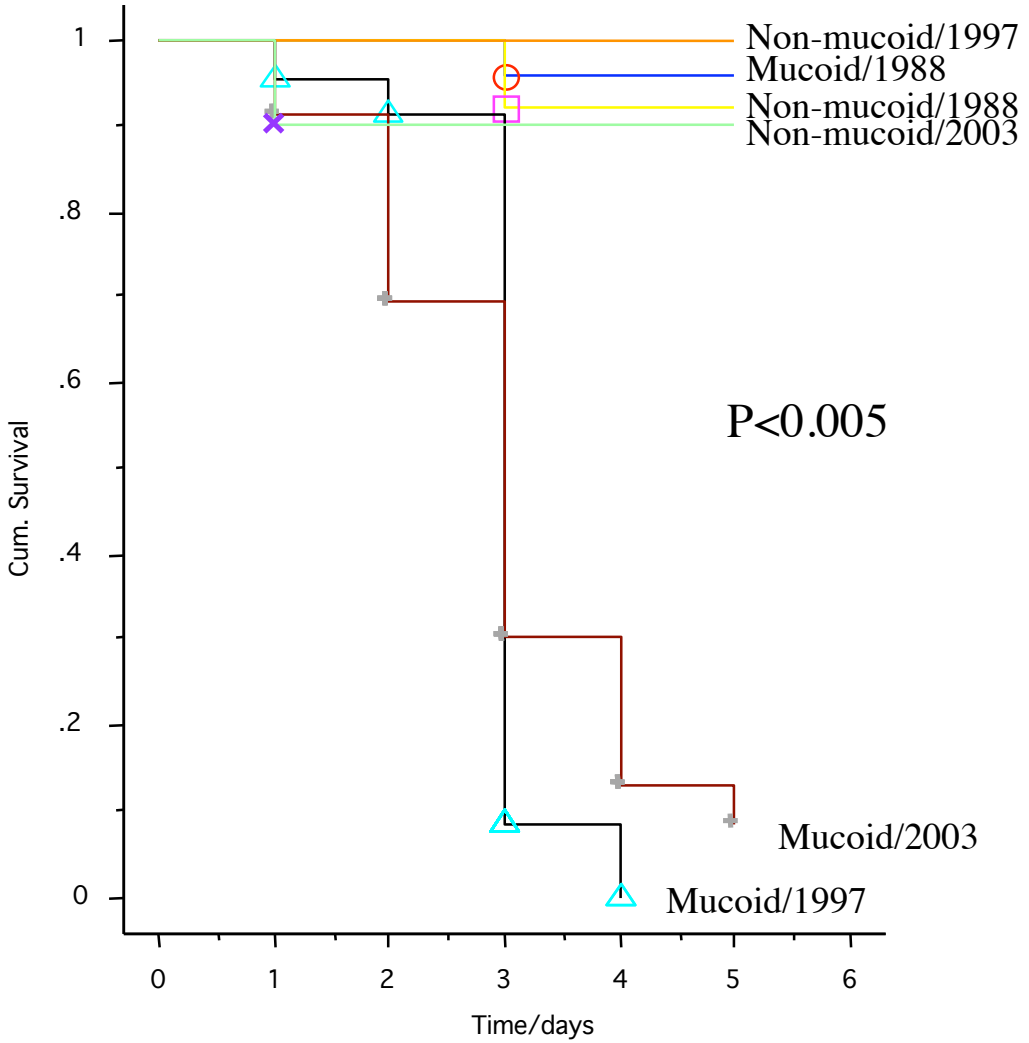
For non-mucoid isolates:

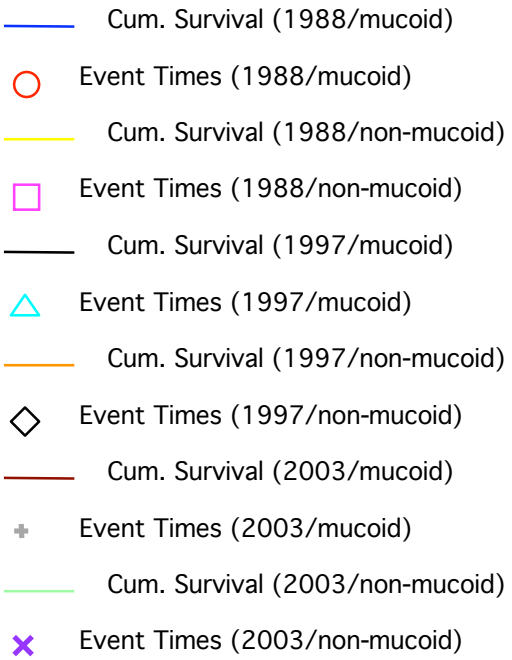
1) Initial stages: Ability for **biofilm formation** correlates to pathogenecity.

2) Late stages: Virulence dominated by other factors - hyperproduction of exopolysaccharides?

	1988	1997	2003	
Non-mucoid	+	+	+	Sacrifize day 5, or day 1, 2 or 3.
Mucoid	+	+	+	

Survival of mice infected with mucoid or non-mucoid *P. aerugionsa*.





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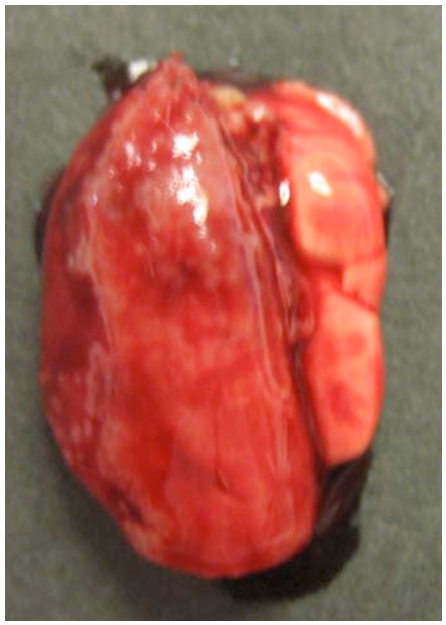
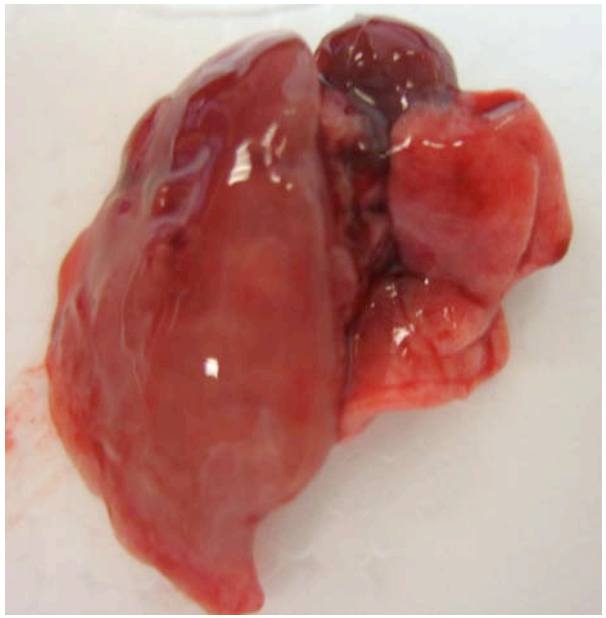
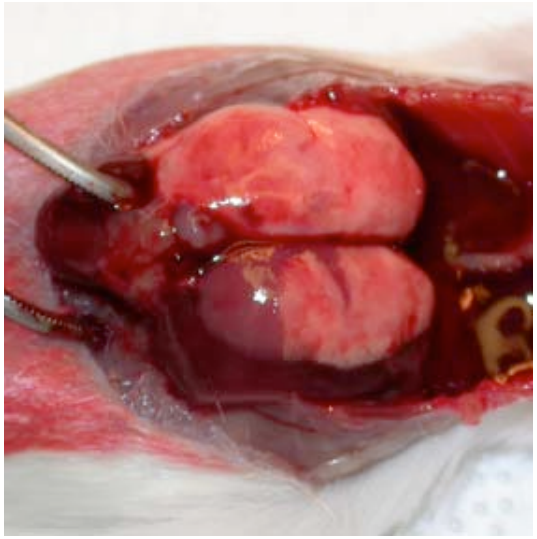
Macroscopic pathology (mucoid ”adaptive” strain).



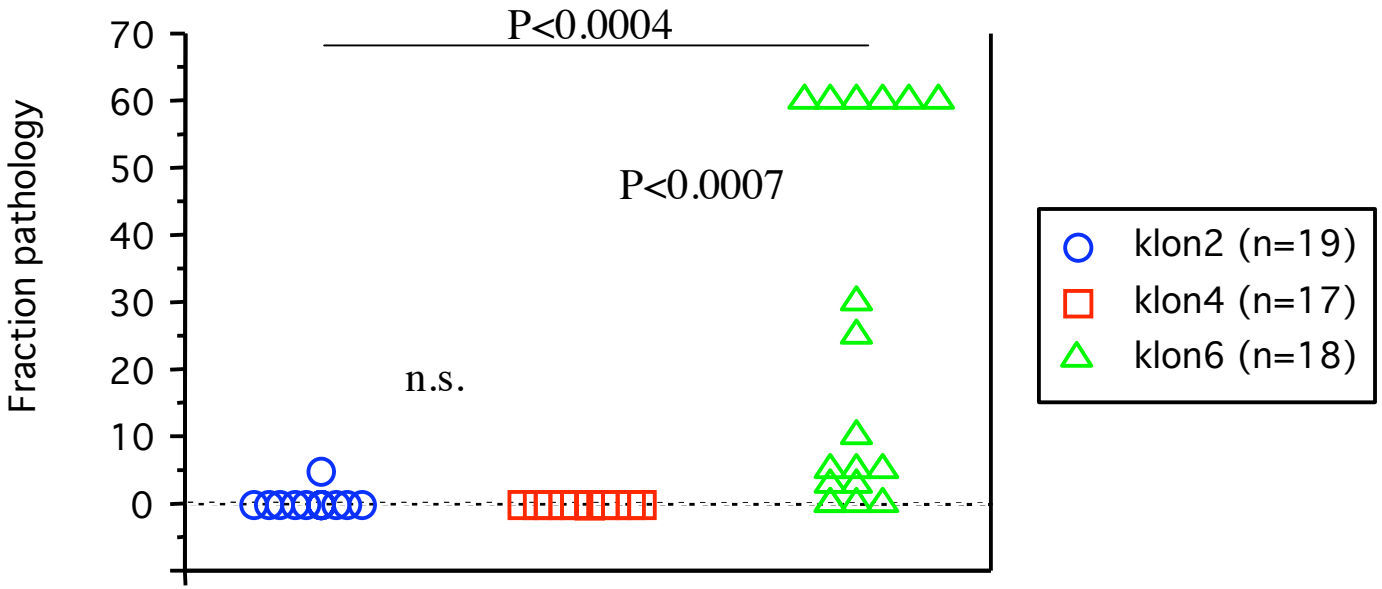
- Athelectasis
- Haemorrhages
- Abscesses
- Adherences
- ”Airtrapping”





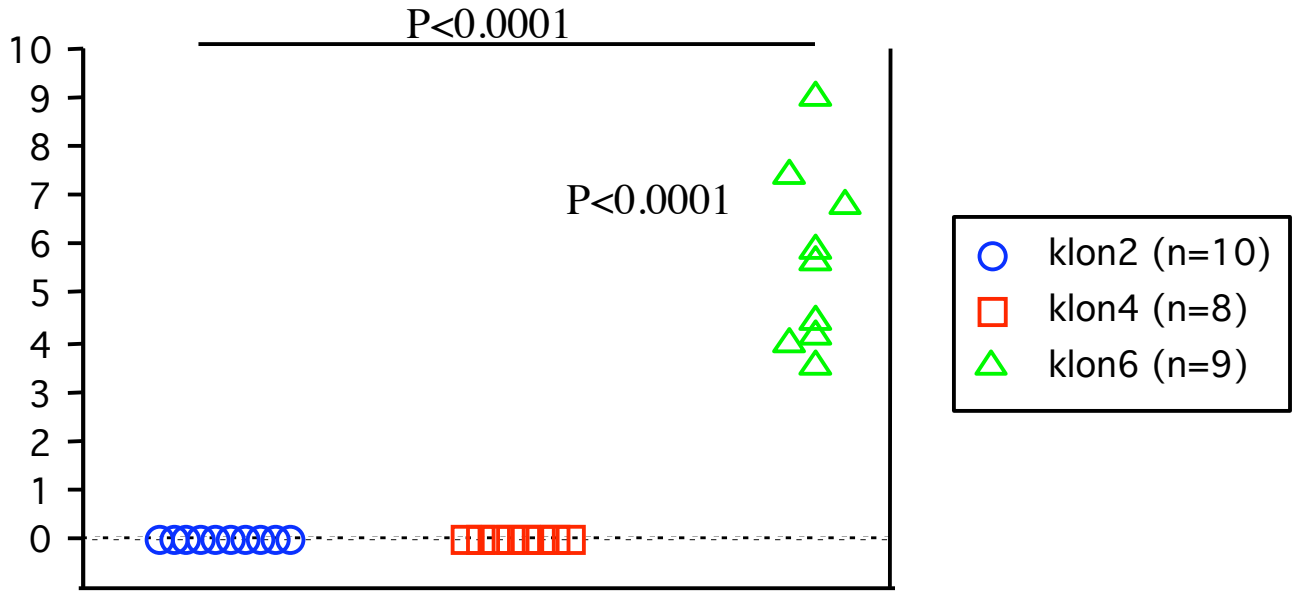
2009

Macroscopic pathology



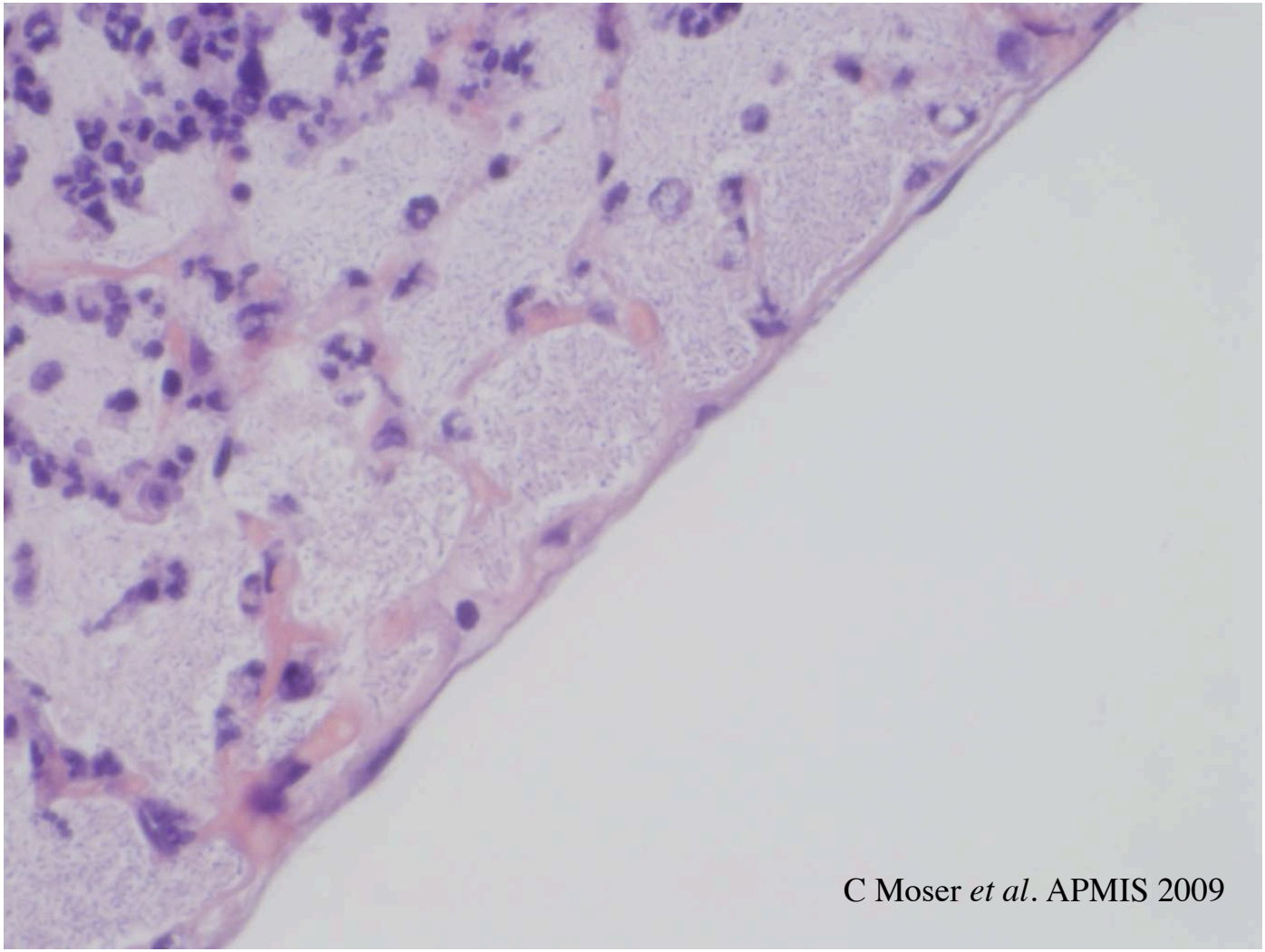
C Moser *et al*. APMIS 2009

Quantitative bacteriology



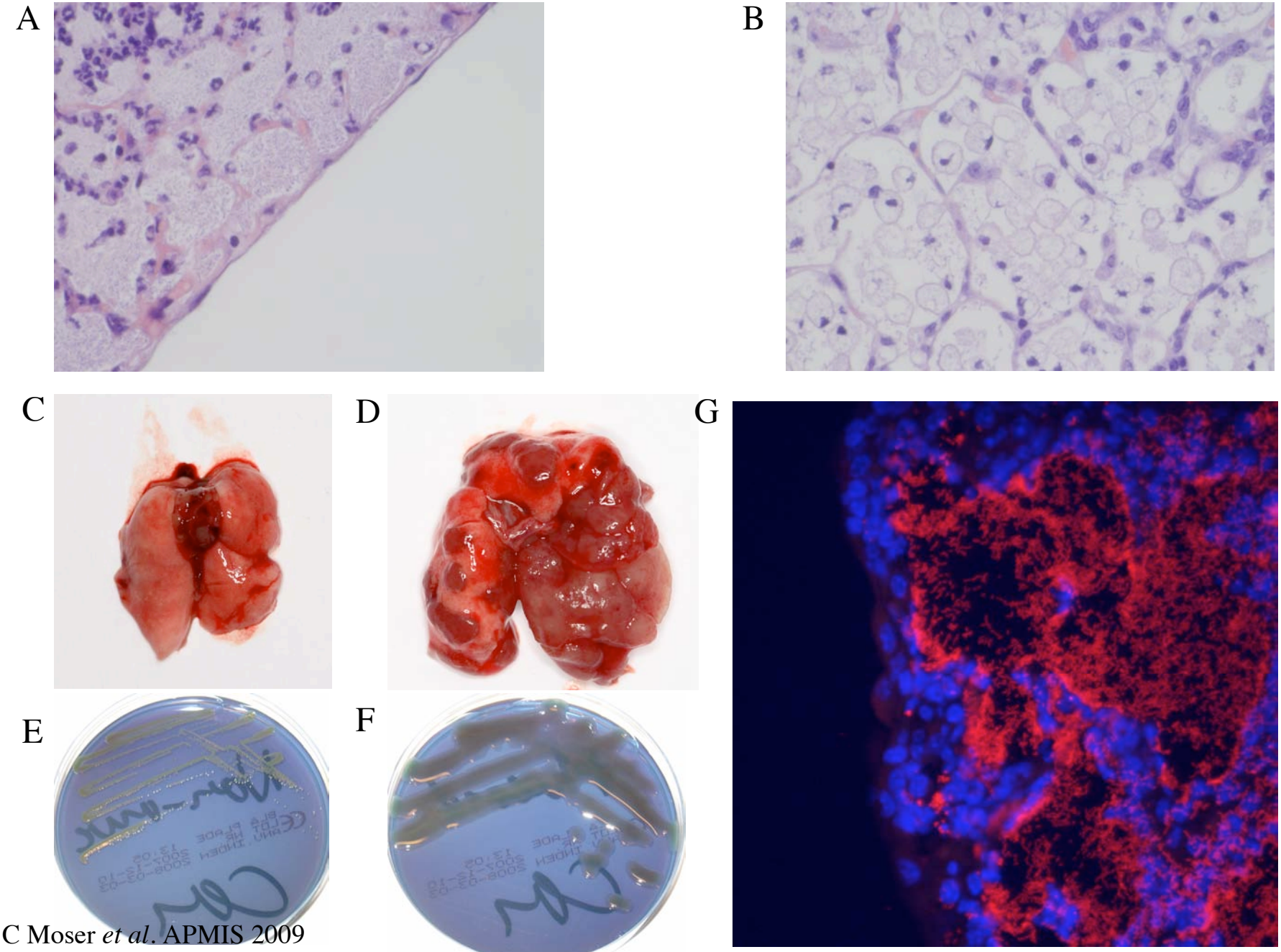
C Moser *et al*. APMIS 2009

Histopathology adaptation



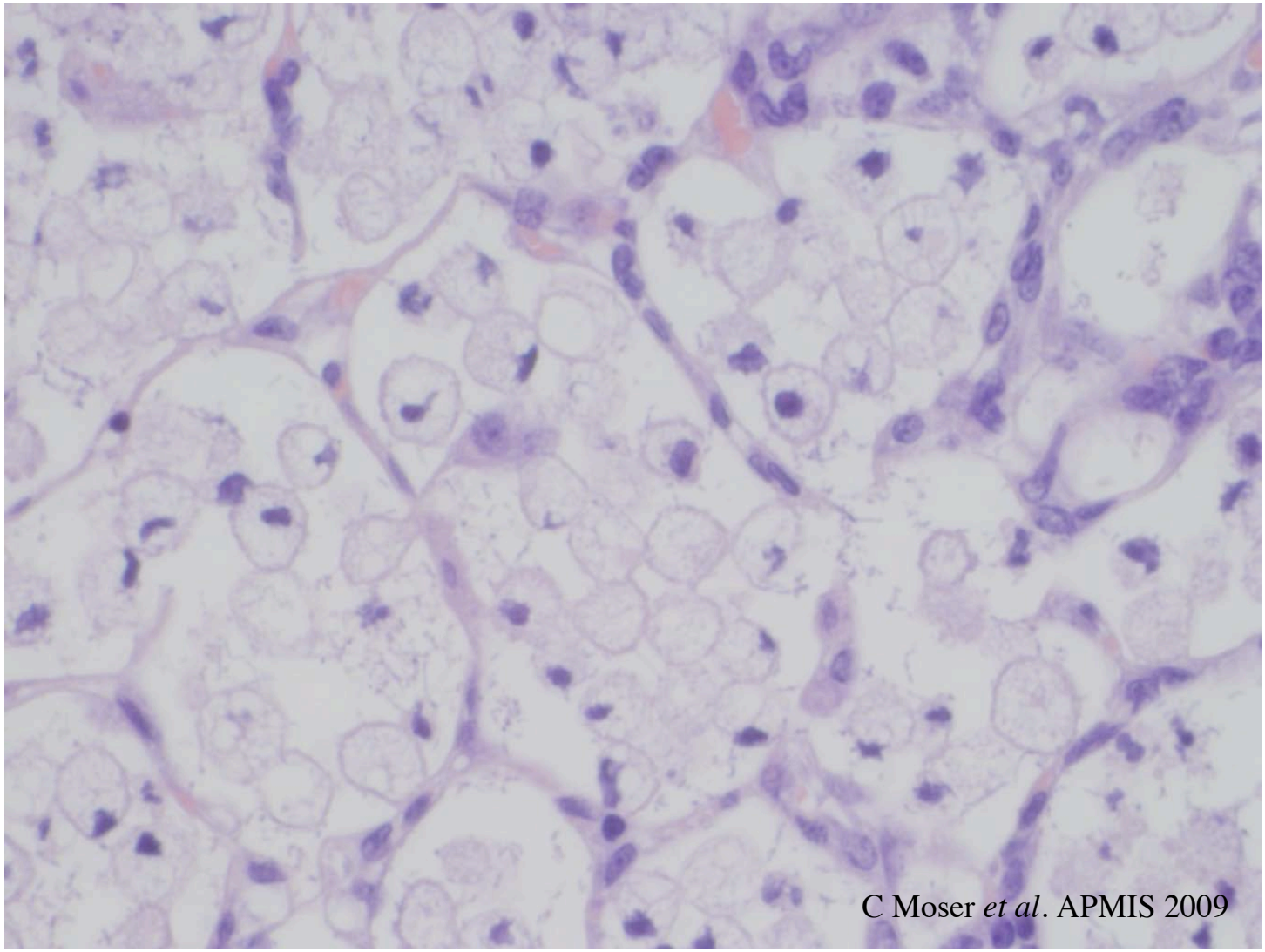
2009

Figure 3. Pathology and colony morphology.



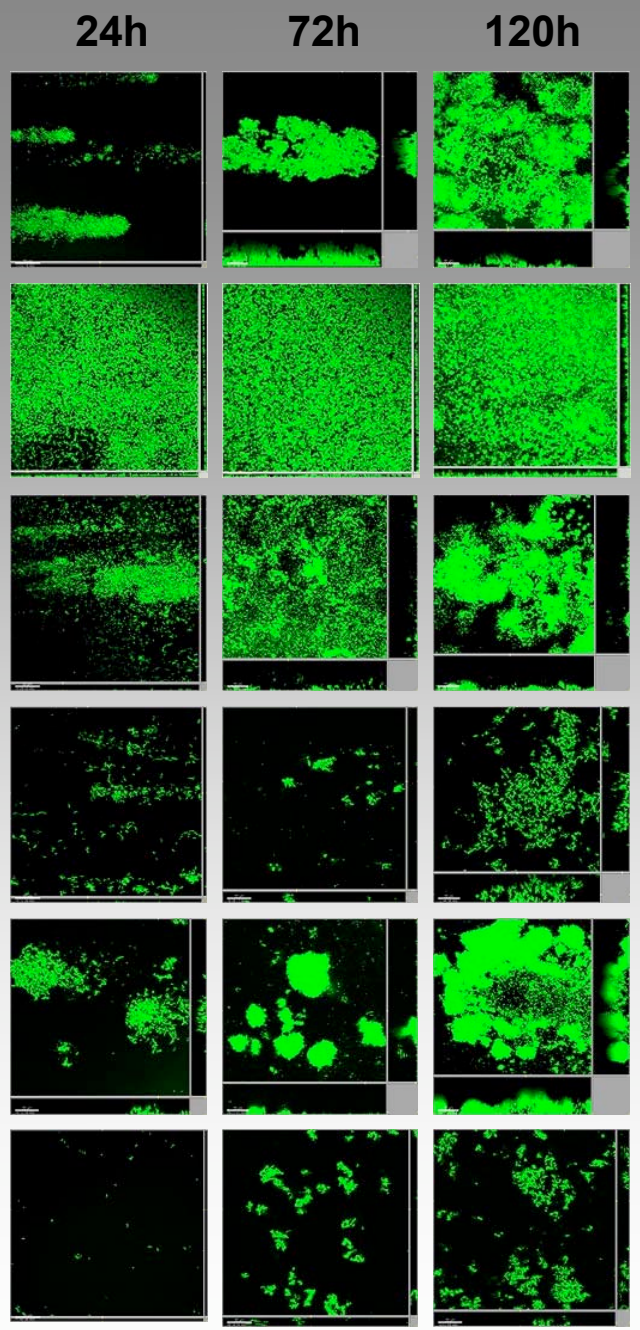
C Moser *et al*. APMIS 2009

Histopathology adaptation



2009

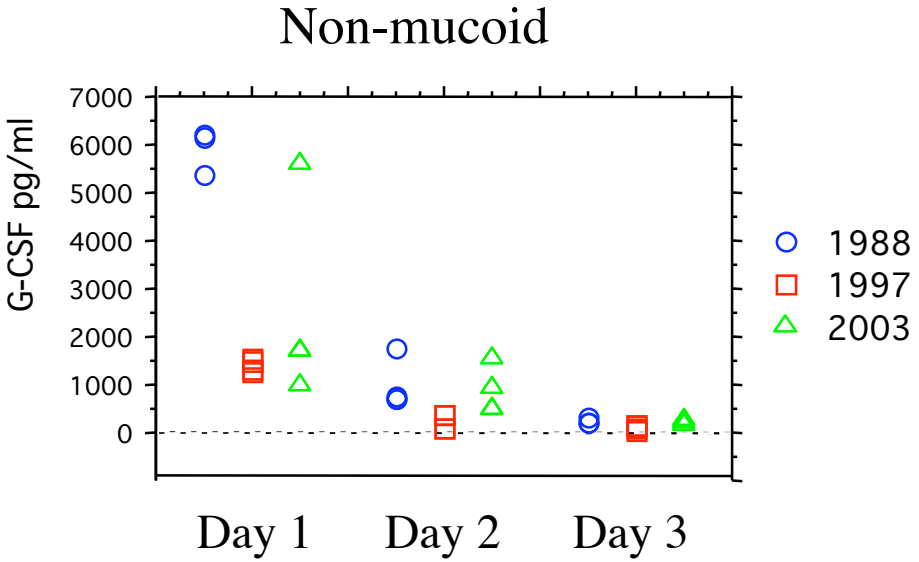
Patient D



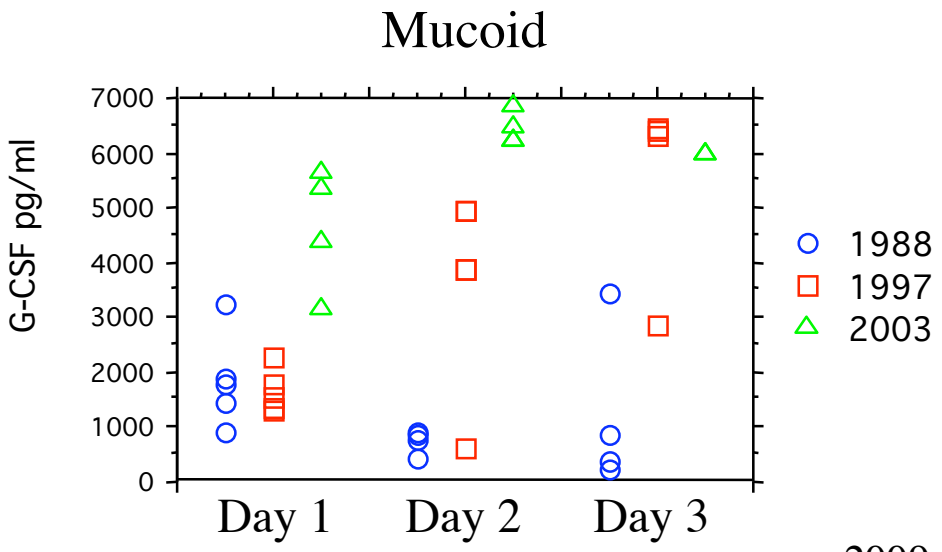
Baoleri Lee. 2006

Inflammatory respons

Non-mucoid versus mucoid.



G-CSF: PMN mobilizer from the bonemarrow

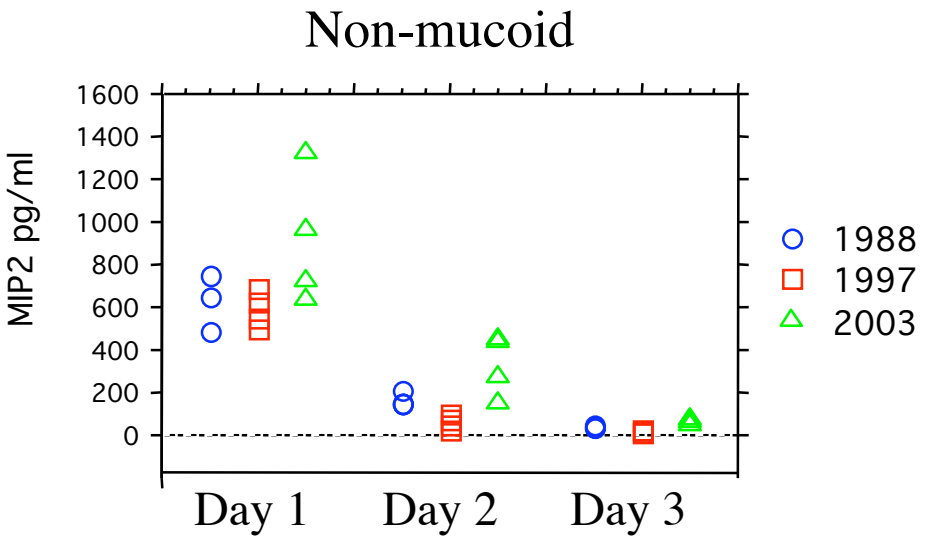


M van Gennip, C Moser et al. Submitted 2009.

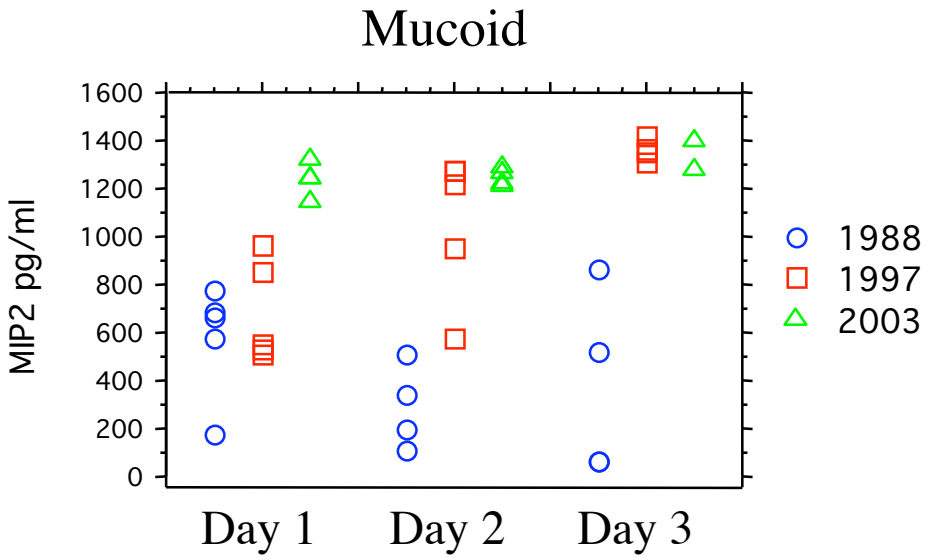
2009

Inflammatory respons

Non-mucoid versus mucoid.



MIP-2: PMN chemoattractant



M van Gennip, C Moser et al. Submitted 2009.

2009

Conclusion adaptation model

- **For non-mucoid isolates:**
  - Initial stages: Ability for biofilm formation correlates to pathogenecity.
  - Late stages: Virulence dominated by other factors - hyperproduction of exopolysaccharide.
- **For mucoid isolates:**
  - More CF-relevant pathology.
  - Virulence increases with time.
- **Combined:**
  - New infection strategy providing a CF-relevant time-perspective (years).
  - A concept that makes it possible to investigate the impact of bacterial adaptation on host responses.

Whole body plethysmography

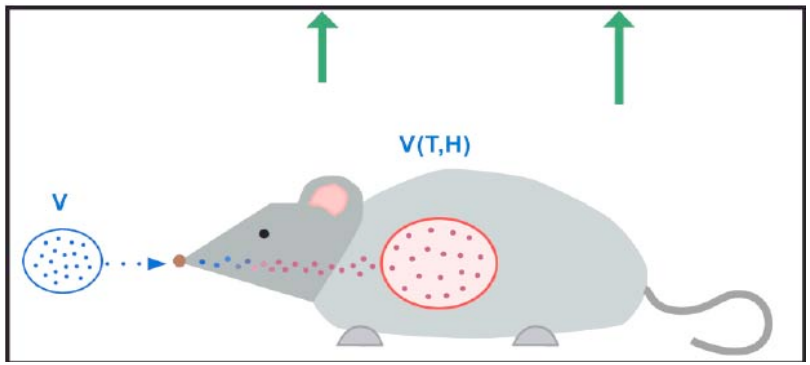
2009

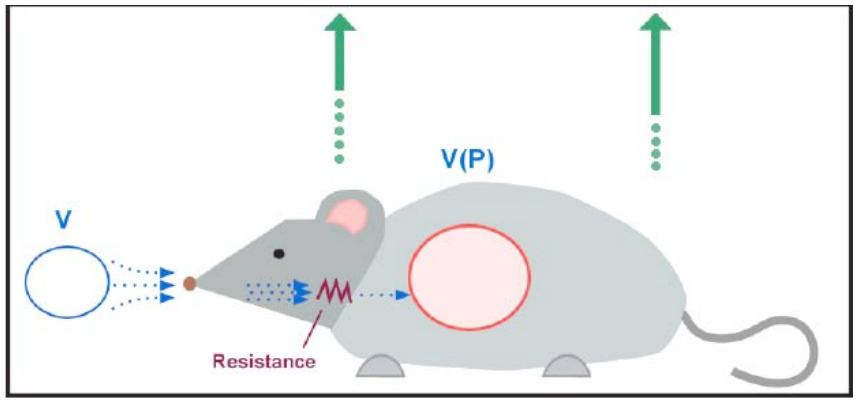
What we measure ?

- The ”box” flow :

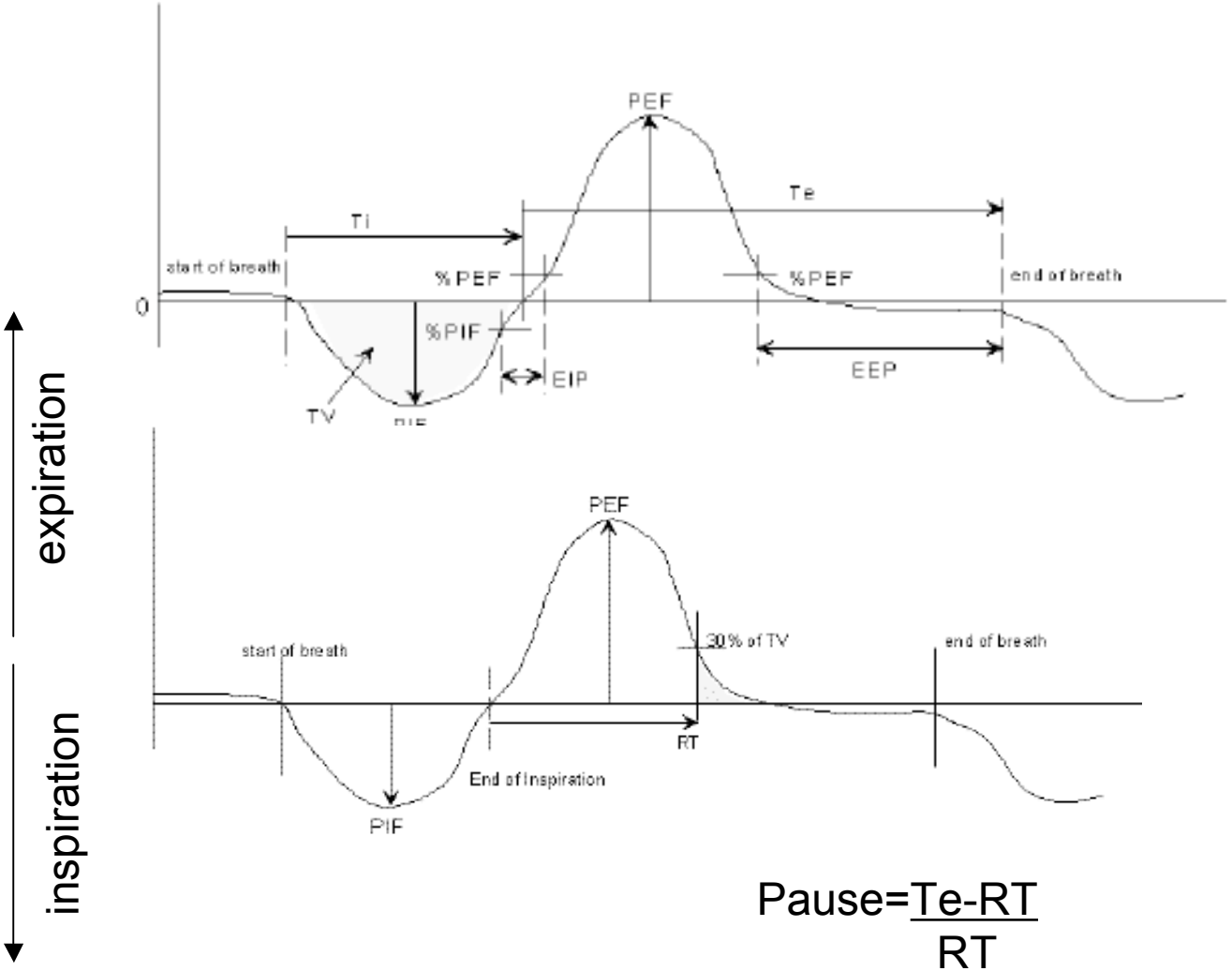
Nasal flow:
conditioning factor
(temp., humidity)

Chest flow:
resistance factor
(negative pressure)





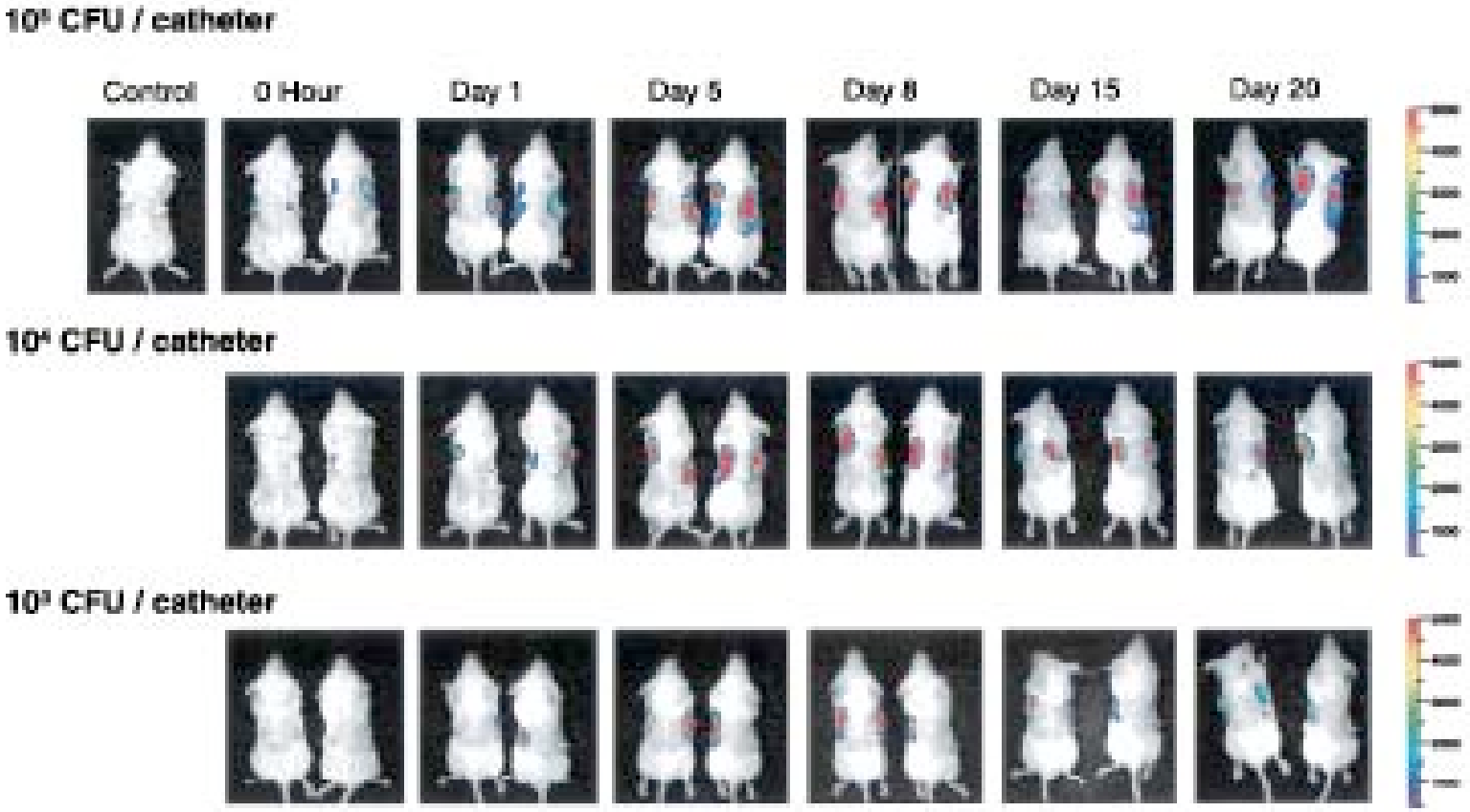
Parameter derivations



$$\text{Pause} = \frac{Te - RT}{RT}$$

$$\text{Penh(Enhanced pause)} = \frac{PEF}{PIF} \times \text{Pause}$$

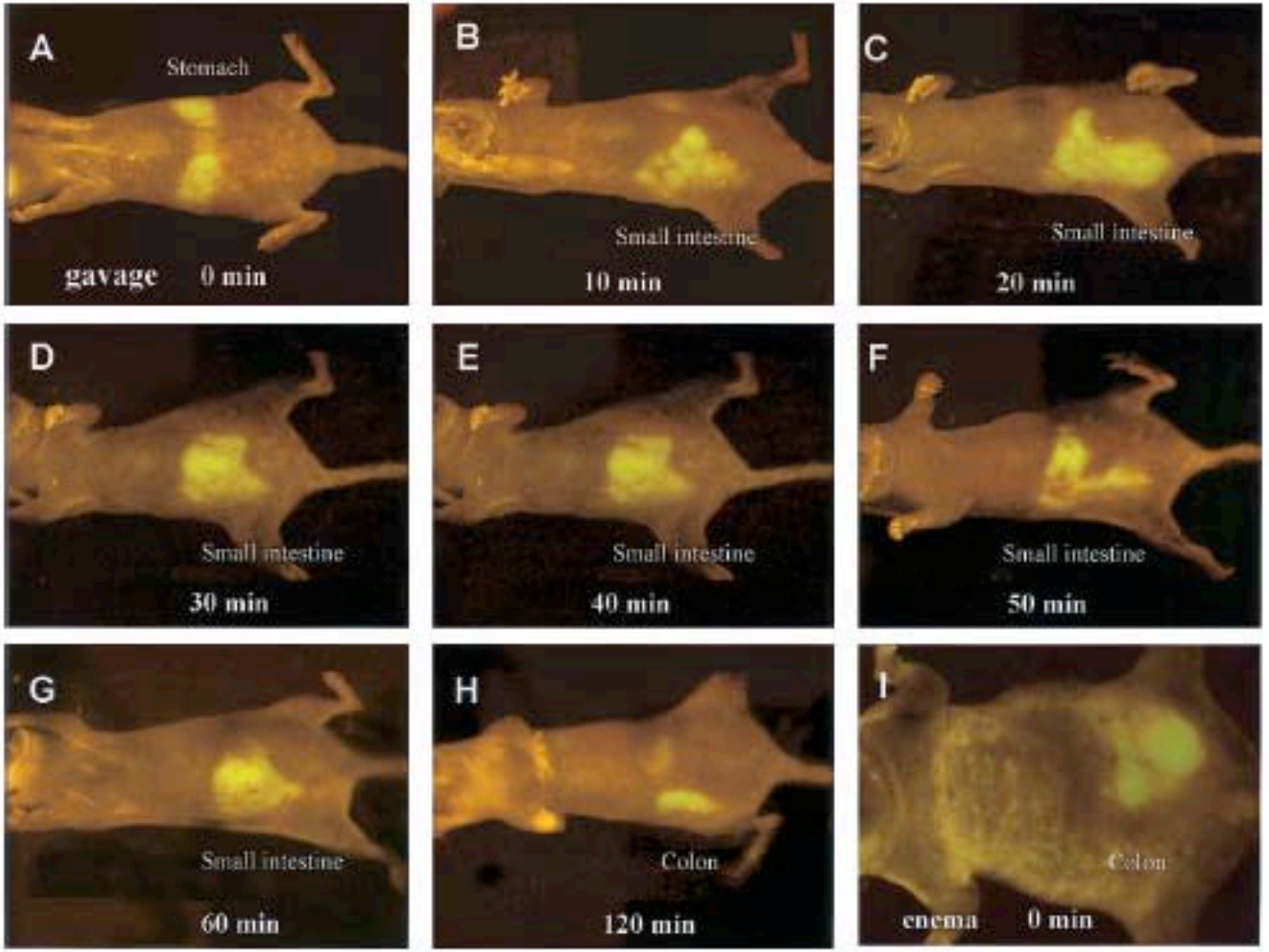
Monitorization of biofilm infection in a mouse model (IVIS imaging system)



Precolonized catheters with *P.aeruginosa Xen 5* were implanted at subcutaneous sites. Makes the bacteria bioluminescent by insertion of a complete *lux* operon.

(Kadurugamuwa, J. Infection and Immunity, vol 71, 882-90, 2003)

E. coli –GFP 10^{11} Light box with blue filter optics.
Hamamatsu three-chip cooled color charge-coupled device camera.



(Zhao et al. PNAS, vol.98, 9814-18, 2001)

Take home messages

- Biofilm infections are numerous, especially in the hospitals, and they are a daily challenge.
- Representative (animal) models are mandatory.
- Several animal models are available. Constantly being improved or should be adjusted to relevant problem being investigated.
- Frequent contact between the clinical world and the basic science is important.

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